BIOCONVERSION PROCESS OF SOURCE-SEPARATED ORGANIC WASTE FOR ETHANOL PRODUCTION

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ABSTRACT

Production of biofuel such as ethanol from lignocellulosic biomass is a beneficial way to meet sustainability, energy security, and environmental goals. Lignocellulosic biomass such as source-separated organic (SSO) waste is particularly attractive since it is widely available, often at a negative cost, reduce the land depletion from using food-based biomass for ethanol production and reduce the amout of generated waste. Therefore, in order to meet the future fuel demands and cope with increasing volume of municipal waste this study was a first attempt to use SSO as a feedstock for ethanol production.

The main objectives of the study were: a) to compare standard and modified cellulose-organic-solvent-based lignocellulosic fractionation (COSLIF) pretreatment of SSO waste for ethanol production in terms of enzyme savings, sugar formation and ethanol yields; b) to produce ethanol from SSO by using modified COSLIF pretreatment and fermentation with two different recombinant strains: *Z. mobilis* 8b and *S. cerevisiae* DA2416; and c) to develop experimental kinetic model capable of predicting behavior of batch SSCF on SSO waste with different SSO substrate concentrations using Berkeley Madonna program.

Based on the obtained results, it was found that SSO is an excellent feedstock material for ethanol conversion. The efficiency of modified COSLIF pretreatment was improved by 20% compared to standard method using ethanol washing of pretreated SSO samples during the experimental procedures instead of acetone. On average, glucose yield from SSO samples pretreated by modified COSLIF was about 90% compared to 10% for untreated samples. *S. cerevisiae* DA2416 outperformed *Z. mobilis* 8b on ethanol yields during the fermentation process, with 0.50 g ethanol/g potential sugar fed on SSO in less than 5 days, with a 96% cellulose conversion, totalling in 150 g/L ethanol produced. A kinetic model with newly integrated values of experimentally defined SSO feedstock constants was proven to predict the ethanol yield accurately with substrate concentration ranges of 20 g/L - 50 g/L. Model prediction at higher substrate concentration (e.g. 100 g/L) deviated from the experimental values, suggesting that ethanol inhibition is a major factor in bioethanol conversion.

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LIST OF ABBREVIATIONS

AIL	Acid Insoluble Lignin
ASL	Acid Soluble Lignin
ASTM	American Society for Testing and Materials
ATS	Aufbereitungs Technology and System, AG
CBP	Consolidated Bio-Processing
COSLIF	Cellulose- and Organic Solvent-based Lignocellulosic Fractionation
DP	Degree of Polymerization
DSA	Dilute Sulfuric Acid
EG	Endo-Glucanase
FPU	Filter Paper Unit
gdcw	Gram dry cell weight
HMF	Hydroxymethylfurfural
MSW	Municipal Solid Waste
NREL	National Renewable Energy Laboratory
ODW	Oven Dry Weight
OFMSW	Organic Fraction of Municipal Solid Waste
SEM	Scanning Electron Microscope
SHF	Separate or Sequential Hydrolysis and Fermentation
SSCF	Simultaneous Saccharification and Co-Fermentation
SSO	Source-Separated Organic
SWM	Solid Waste Management
TAPPI	Technical Association of the Pulp and Paper Industry

TKN	Total Kjeldahl Nitrogen
TS	Total Solid
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound
VS	Volatile Solid
EJ	Exajoule

CHAPTER 1. INTRODUCTION

1.1. Biofuel from Lignocellulosic Conversion

Over the last century as the world population has grown and more countries have become industrialized, energy consumption has increased steadily. Therefore, there is a great interest in exploring alternative energy sources. Unlike fossil fuel, ethanol is a renewable energy source produced via fermentation of sugars from various biomasses. Nowadays, ethanol is produced from both: food based biomass such as corn/sugarcane/wheat (Shen et al., 2012; Ferreira et al., 2014; Mohagheghi et al., 2015), and non-food based materials such as lignocellulosic biomass (Wyman and Yang, 2009; Zhao et al., 2012; Roberts et al., 2015).

Ethanol production using the current corn/starch based technology may not be practical because corn production for ethanol will compete with food for the limited agricultural land needed. Therefore, a potential source for low-cost ethanol is to utilize lignocellulosic biomass. Common examples of lignocellulosic biomass include energy crop, waste from agriculture and forest products, industries, and municipal waste. Unprocessed lignocellulosic biomass typically contains the order of 35%-50% cellulose, 20%-35% hemicellulose, and 15%-20% lignin plus extractives and ash (Wyman, 1999).

Figure 1.1 shows main constituents of lignocellulosic biomass in which cellulose is the primary component. The backbone of cellulose consists of D-glucose subunits bonded by β-1-4 linkages. This structure is stabilized by inter-chain hydrogen bonding between the G-3 hydroxyl and the oxygen in the pyranose ring. Hemicellulose, on the other hand, consists of branched chains of several sugar subunits such as xylose, mannose, arabonose, and galactose. It bonds to the cellulose and lignin molecules through covalent and hydrogen bonds (Fan et al., 1987).

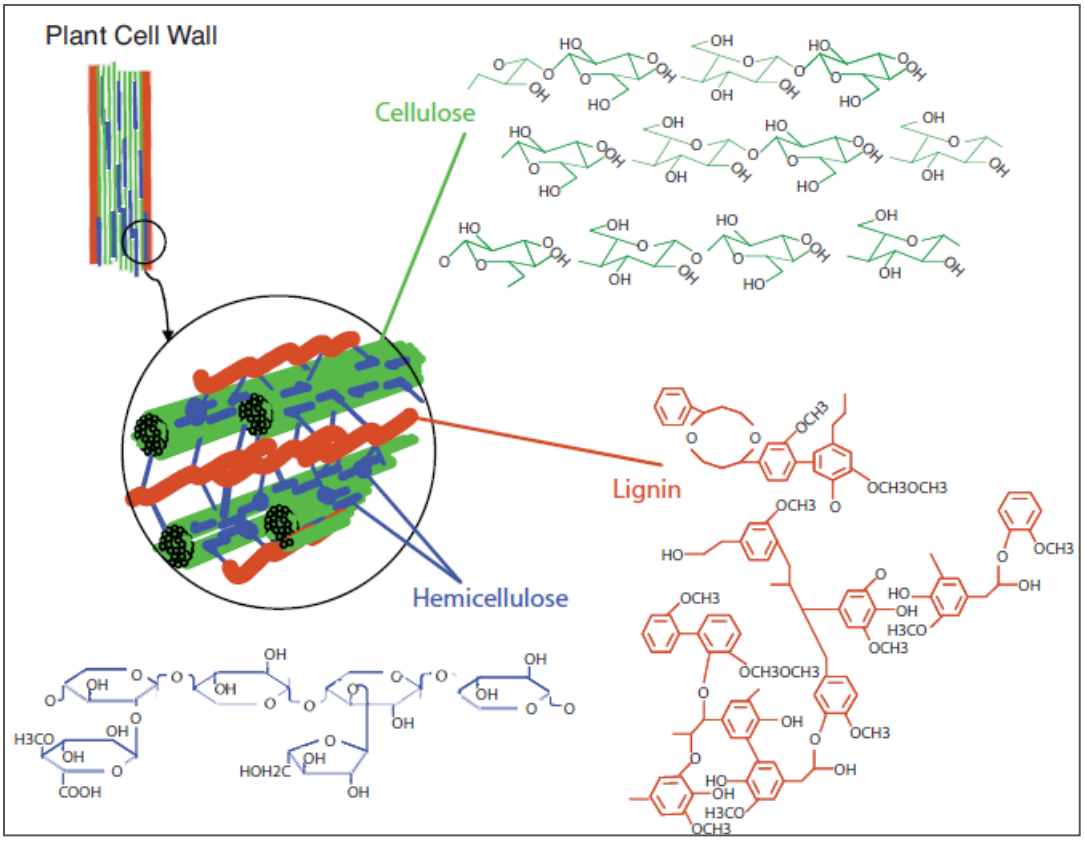


Figure 1.1: Lignocellulosic biomass constituents

Source: Genome Management Information System,Oak Ridge National Laboratory (2014)

Lignin is a very complex aromatic polymer composed of polyphenolic compounds arranged in branched chains. Lignin intermixes with cellulose and hemicellulose to form a matrix in the plant cell wall, providing strength to the plant and increasing its resistance to biodegradation (Wiselogel et al., 1996). Lignin from biomass can also be gasified to release the heat value contained to provide energy needed for conversion processes (Johansson et al., 1993).

Extensive research has been completed in the past on conversion of lignocellulosic biomass to ethanol (Dale et al., 1984; Cadoche and Lopez, 1989; Duff and Murray, 1996; Wright, 1998; Zhang and Lynd, 2010; Sathitsuksanoh et al., 2012; Bayens et al., 2014). As

shown in Figure 1.2, processing lignocellulosic biomass to ethanol usually consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation.

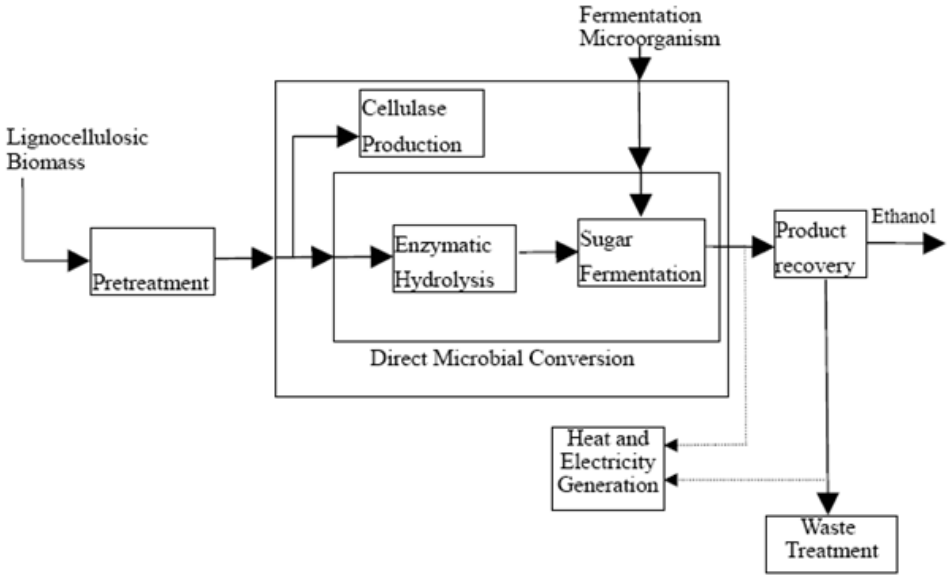


Figure 1.2: Major steps in conversion of lignocellulosic biomass to ethanol

The effect of pretreatment of lignocellulosic biomas has been recognized for a long time (McMillan, 1994). The purpose of pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the material so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. Although a variety of process configurations have been studied for conversion of lignocellulosic biomass into an ethanol, enzymatic hydrolysis is deemed more competitive compared to other liquid fuels on a large scale (Wyman, 1999).

Cellulose can be hydrolytically broken down into glucose either enzymatically by cellulases, or chemically by sulfuric or phosphoric acids. Hemicellulases or acids hydrolyze the hemicellulose polymer to release its component sugars. Glucose, galactose, mannose, and six carbon sugars are readily fermented to ethanol by many naturally occuring organisms. On other

hand, only very few native strains ferment five carbon sugars such as xylose and arabinose and usually at relatively low yields. Xylose and arabinose generally comprise a significant fraction of hardwoods, agricultural residues, and grasses. Table 1.1 summarizes the composition of most common lignocellulosic feedstocks. It maybe observed that typical carbohydrate contents range from 31%- 81% by dry weight, and they stand from cellulose and hemicellulose.

Table 1.1: Percent dry weight composition of lignocellulosic feedstocks

Feedstock	Carbohydrate	Carbohydrate	Non-carbohydrate
	Glucan (cellulose) %	Xylan (hemicellulose) %	Lignin %
Corn stover	36.4	18.0	16.6
Corn fiber	14.28	16.8	8.4
Pine wood	46.4	8.8	29.4
Popular	49.9	17.4	18.1
Wheat straw	38.2	21.2	23.4
Switch grass	44.9	31.4	17.6
Office paper	68.6	12.4	11.3

Source: Lynd et al., 1999

Cellulose and hemicellulose are potential sources of fermentable sugars (Ho et al., 1998; Taherzadeh et al., 1999; Sreenath and Jeffries, 2000). The presence of lignin in the cell wall, however, hamper enzymatic hydrolysis of the carbohydrates. The relationship between structural and compositional factors reflect the complexity of lignocellulosic materials. The variability in these characteristics accounts for the varying digestibility between different sources of

lignocellulosic biomass. In general, effective pretreatment causes disruption of these barriers and prepares for enzymatic hydrolysis folowed by fermentation (Lynd et al., 1991; Holtzaplle, 1993; Mosier et al., 1999).

According to the cost analysis done by Lynd et al. (1999), among the various process steps, pretreatment and biological processing accounts for 32.7% and 39.6% respectively of total processing costs, which means that improvements are likely to reduce the the cost of ethanol production in both operational units to the level competitive with the cost of fossil fuels.

1.2. Innovations in Biotechnology of Fuels and Cellulosic Feedstock Production

Biotechnology of fuels is a versatile field with a goal to develop an alternative energy source. It stimulates research for alternative sources of energy via bio-chemical conversion processes. General movement is utilizing substantial amount of lignocellulosic biomass readily available for little cost. The earth has a huge stock biomass covering wide regions including forests and the ocean. The total biomass of the world is 1,800 billion tons on the ground and 4 billion tons in the ocean, and a comparative amount of biomass exists in the soil (The Asian Biomass Handbook, 2008). The total biomass on the ground is 33,000 EJ on the energy basis, which corresponds to 80 times or more of the annual energy consumption of the world. However, it is difficult to know the amount of waste biomass production in each country and region of the world. Therefore, the waste biomass production is often estimated typically by assuming ratio of waste production relative to the biomass resources production (The Asian Biomass Handbook, 2008). An example of the estimation of waste biomass production is shown in Table 1.2.

Table 1.2: Estimation of waste biomass production and amount of resources

Biomass species	Ratio of waste production (t/t)	Coefficient of energy conversion (GJ/t)
Rice	1.4	16.3
Wheat	1.3	17.5
Maize (corn)	1.0	17.7
Roots and tubers	0.4	6.0
Sugarcane residue (tops and leaves)	0.28	17.33
Cattle	1.10 (t/y/head)	15.0
Swine	0.22 (t/y/head)	17.0
Poultry	0.037 (t/y/head)	13.5
Horses	0.55 (t/y/head)	14.9
Buffaloes and camels	1.46 (t/y/head)	14.9
Sheep and goats	0.18 (t/y/head)	17.8
Industrial logs	1.17	16.0
Fuel logs	0.67	16.0
Wood waste	0.784	16.0

Source: The Asian Biomass Handbook, 2008

The current stock of biomass is estimated based on waste biomass production multiplied by a coefficient of energy conversion. It is estimated at approximately 43 EJ for livestock biomass, 48 EJ for agricultural biomass, and 37 EJ for forestry biomass, with totals of approximately 128 EJ.

Approximately 22 EJ of dung of cattle accounts for the largest part of resources, which is followed by an approximately 20 EJ of log residue.

Conversion of lignocellulosic biomass to valuable biofuels has received the largest attention in past two decades and has concentrated on three main transformations: photosynthetic feedstock production; reduction of recalcitrance of biomass involving breakdown of complex components into simpler sugars; and end-product formation from sugars (Lynd, 2008). A number of studies indicate lignocellulosic feedstock could attain high yields (mainly in the form of ethanol) with positive balance in investment return (Lynd, 2008; Wyman, 2008). In Canada and US, for example, the goal is to provide more alternative greener fuels into the market and decrease the prevailing fossil fuel utilization. A conservative estimate places lignocellulosic crops at $50-$60 per metric ton (raw biomass), or converted to the price per energy value at $3/GJ, which is equivalent to the value of crude oil at the $17 per barrel (Lynd et al., 2008). There is a good potential for development of lignocellulosic fuels, but current implementation is still slow. This is because of the established corn ethanol alternative and due to retracted market for ethanol compared to gasoline (Bullis, 2013). However, there is a positive sign for a brighter future of lignocellulosic biomass, which eventually is expected to become a valuable product through government policy changes (Tyner, 2011) and improvements in new advances in the biotechnology realm.

1.3. Source Separated Organic Waste as a Promising Cellulosic Feedstock

While today ethanol is typically produced from the starch contained in grains such as corn, sugarcane, and grain sorghum, it also can be produced from cellulose which is mainly

present in non-food products. Cellulose is the main component of plant cell walls and it is the most common organic compound on earth, which may be converted into usable sugars for ethanol production. Cellulosic ethanol is a blend of normal ethanol that can be produced from a great diversity of biomass including waste from urban, agricultural, and forestry sources.

Biomass such as processed source- separated organic (SSO) waste is particularly attractive in one context since it is widely available at negative cost and has many other benefits (e.g., good alternative fuel in terms of greenhouse gas (GHG) emissions, reduces of farm land's depletion, diminutive of generated waste) and life-cycle settings. Generally, SSO sample prepared for this study is a blend of approximately 78%-80% of organic green bin waste 20%-22% of construction and demolition waste in form of wood chips, passed through the thermal screw press (TSP) (Vartek Waste Management Ltd, 2005). A type of the woodchips chosen may vary (Sims, 2004; EUBIA, 2007) and can be any kind of woody or agricultural waste as presented in Table 1.3, which may alter the composition.

The composition of biomass may vary depending on several local factors: 1) sorting criteria specified by the municipality for use by the households; 2) efficiency of the citizens in sorting properly; 3) collection system including the types of collection bags used in the kitchen (paper, plastic) and local storage bins (containers, paper sacks), in the so-called "green bin program", and finally 4) pretreatment that is used (disc screen, screw separator, or magnetic separator) prior to the biological treatment (la Cour Jansen et al., 2004).

Table 1.3: Classification of biomass resources

Supply sector	Type	Example
Woody Biomass	Dedicated forestry	Short rotation plantations (e.g. willow, poplar, eucalyptus)
	Forestry by-products	Wood blocks, wood chips from thinning
	Wood process residue	Bark, sawdust, shavings, wood chips and off-cuts
	Recovered wood fuels	Recovered wood fuels from activities such as land clearance and municipal green waste
Agriculture	Dry lignocellulosic energy crops	Herbaceous crops (e.g. miscanthus, reed canarygrass, giant reed)
	Energy crops short rotation and annuals	Oil seeds for methylesters (e.g. rape seed, sunflower) Sugar crops for ethanol (e.g. sugar cane, sweet sorghum) Starch crops for ethanol (e.g. maize, wheat)
	Agricultural residues	Straw, pruning from vineyards and fruit trees
	Livestock waste	Wet and dry manure (cattle, pigs, horses and poultry as well as human)
	Agro-industrial by-products	Bagasse, rice husks
	Water vegetation	Algae, water hyacinths, seaweeds
Industry	Industrial residue	Industrial waste wood, sawdust from sawmills Fibrous vegetable waste from paper industries
Waste	Dry lignocellulosic	Residues from parks and gardens (e.g. prunings, grass)
	Contaminated waste	Demolition wood Organic fraction of municipal solid waste Biodegradable landfilled waste, landfill gas Sewage sludge

Source: Sims, 2004; EUBIA, 2007

1.4. Ethanol Prediction Yields

Four approaches for biomass process configuration featuring enzymatic hydrolysis and fermentation have been reported in the literature (Lynd et al., 2002): separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bio-processing (CBP).

Depending on the type of biomass/feedstock and process configuration (SHF, SSF, SSCF, CBP), actual yield of the ethanol could be anywhere from 60% to 90% of theoretical (Dowe and McMillan, 2008). According to Dowe (2009) the stoichiometric maximum theoretical yield of ethanol is concluded at 0.51 g ethanol / g glucose or xylose. Theoretical yield of greater than 90% from glucose is achievable by robust fermentative organisms such as *Saccharomyces cerevisiae* and *Zymomonas mobilis* (Chu and Lee, 2007).

Currently estimated yields from cellulosic biomass are expected to increase as conversion technologies mature with greater flexebility to accommodate differenet feedstock compositions. It is clear that lignocellulosic biomass offers an opportunity for energy production but there are many social, political, economic and environmental conditions that affect the scale of this production. Since none of these conditions are static, there is unlikely to be a definitive calculation for the amount that can be produced. In the rough estimation, the international consensus summarized in the IPCC Special Report on Renewable Energy sources and Climate Change Mitigation (SRREN) that 100EJ – 300EJ per year can be achieved from biomass by 2050 (Davis et al., 2014).

1.5. Selection of Microorganisms

Microorganisms for ethanol fermentation are typically evaluated in terms of the following performance parameters: ethanol yield, inhibitor tolerance, substrate productivity, growth rate, pH, and temperature range. Currently, attempts to develop microorganisms are primarily focused in two areas: a) developing universal microorganisms that can utilize various sugars; and b) developing microorganisms for the specific processing configuration (SHF, SSF , SSCF, CBP). Generally, there are two strategies for developing microorganisms to enhance the range of sugars used. The first strategy considers microorganisms that can utilize different substrates. It is called the “recombinant substrate utilization strategy”. The second strategy is the so-called “native substrate technology”, which implies microorganisms that naturally use multiple sugars and enhance their ability to produce ethanol (Lynd et al., 1999).

A number of different microorganisms have been employed in the fermentation of the water-soluble lignocellulose derived hexose sugars to ethanol. For the most part, the yeast *Saccharomyces cerevisiae* has been used for fermentation.

To overcome the concerns with pentose sugars fermentation, some researchers have explored the use of alternative microorganisms such as *Pichia stipitis, Pachysolen tannphilus,* and *Candida shehatae* (Jeffries, 1983; Hahn-Hagerdal et al., 1993). These microorganisms can ferment xylose to ethanol, but have low productivity and ethanol yield (du Preez et al., 1989; Hahn-Hagerdal et al., 1994a) and increased nutritional and aeration requirements (Dellweg et al., 1984; du Preez et al., 1985; Skoog and Hahn-Hagerdal, 1990). These microorganisms may also show increased sensitivity to ethanol, resulting in inhibition of both growth and fermentation (du Preez et al., 1989). Moreover, they do not metabolize the other sugars such as glucose as fast as *S.cerevisiae* (du Preez et al., 1986; Hahn-Hagerdal et al., 1994b) and maybe more vulnerable to

potential inhibitors presented in water soluble fraction (Linden and Hahn-Hagerdal, 1989; Delgenes et al., 1996).

A series of recombinant *S. cerevisiae* strains that can utilize xylose have been developed in past decades at Purdue University (Moniruzzaman et al., 1997; Ho et al., 1998). Strains of *S. cerevisiae* are capable of high rates of ethanol production from a range of carbon sources, displaying high ethanol tolerance (Ingram, 1986), and increased resistance to potential inhibitor compounds (Martin and Jonsson, 2003). However, a main obstacle of bioconversion with earlier *S. cerevisiae* strains were an inability of microorganisms to metabolize pentose sugars (xylose and arabinose) into ethanol, especially from harwood and agricultural residues. Agricultural residues and hardwoods are similar in the way that they have lower lignin content and the hemicellulose produces significant amounts of pentose sugars. On the other hand, softwoods have a higher lignin content, which makes the hydrolysis step more difficult, but they generally produce less pentose sugars.

More recent development in gene engineering has resulted inrelatively new recombinant microorganisms such as *S. cerevisiae* RWB222 and *S. cerevisiae* DA2416 avilable for biomass conversion with ability to utilize pentose sugars. *S. cerevisiae* RWB222 strain was genetically modified derivative of *S. cerevisiae* CEN, PK. Xylose utilization in this strain was achieved by integration of the xylose isomerase from *Piromyces sp.* E2, over expression of the native pentose phosphate pathway, and directed evolution for growth on xylose (van Dijken et al., 2000; Kyper et al., 2005). The isomerase is vital for *S. cerevisiae* to grow well on xylose under anaerobic conditions with high ethanol production (Maris et al., 2006).

In another study, Suk-Jin Ha et al. (2011) have developed a unique strategy to coferment a mixture of xylose and cellobiose with *S. cerevisiae* DA2416 strain (derivative of *S. cerevisiae*

D452-2). They introduced a newly discovered cellodixtrin transporter and intracellular β-glucosidasefrom cellulotic fungi, *Neurospora crassa* (Galazka et al., 2010) into a *S. cerevisiae* DA2416 strain. It was engineered to ferment xylose with improved ethanol yields of 0.39 g/g from a mixture of cellobiose and xylose as compare to ethanol yields of 0.31-0.33 g/g from fermentation either cellobiose or xylose as sole carbon sources. These results suggest that cofermentation of glucose and xylose can enhance the overall ethanol yields and productivities.

Along with the development of *S. cerevisiae* strains, a series of bacteria *Zymomonas mobilis* that can use xylose and/or arabinose were developed in the National Renewable Energy Laboratory (Zhang et al., 1995; Deanda et al., 1996; Zhang et al., 1998). They illustrated the recombinant substrate utilization strategy. *Z. mobilis* is the native ethanogenic bacterium with a high ethanol yield. This microorganism can produce greater quantities of ethanol per mole of biomass produced due to the Enther-Doudoroff metabolic pathway. However, the natural substrate range of *Z. mobilis* is restricted to the fermentation of glucose, fructose, and sucrose, limiting its applicability for biomass tranformation (Lindsay et al., 1995).

To overcome these shortages, engineered *Z. mobilis* strains were developed (Zhang et al., 1995). These engineered microorganisms were likely intended to be superior in lignocellulosic bioconversion, but commercial application did not take place. Among the further achievements were two engineered *Z. mobilis* strains: *Z. mobilis* 8b (Joachimsthal et al., 1999) and *Z. mobilis* AX101 (Kompala et al., 2001). *Z. mobilis* 8b was developed as an integrant of *Z. mobilis* ZM4 tolerant up to 16 g/L acetic acid and it can grow from pH 3.5 up to pH of 7.0 with optimum pH value range from 5.0 to 6.0 in ethanol production process (Kim et al., 2000). Its rival, *Z. mobilis* AX101, is capable of fermenting both xylose and arabinose effectively, with the yield achiviement of more than 80% of theoretical within 50 hours (Kompala et al., 2001).

In a new study, Mohagheghi et al. (2015) have adapted and evolved *Z. mobilis* strain 8b for enhanced tolerance to the toxic inhibitors present in corn stover hydrolysate. The adapted strain, named SS3, has a higher xylose utilization rate and produces more ethanol than the parent strain, providing foundation for future research directions in improving *Z. mobilis* for ethanol production and other fuel precursors.

Based on todays' market availability and their ability to utilize glucose and xylose sugars from pretreated biomass in the fermentation phase, robustness and tolerance to inhibitors, two different recombinant glucose and xylose utilizing strains, *Z. mobilis* 8b and *S. cerevisiae* DA2416, were chosen for detailed study in the investigation of SSO waste for ethanol production.

1.6. Ethanol production and inhibitors

In the study of ethanol production, many researches (Philippidis et al., 1992; McMillan et al., 1999; Lynd et al., 2001; Kumar et al., 2009; Zhang et al., 2009) have identified and measured certain parameters: maximum specific rates, ethanol inhibitors and yield, pH, and temperatures. In most cases of measuring maximum specific rates, the initial substrate concentartion is kept low (below 10 g/L), so the effect of produced ethanol could be ignored.

The effect of ethanol on maximum specific rate, μ_m^o, is usually described by the non-competitive inhibition equation by van Uden (1989) as in the following:

$$\mu = \frac{\mu_m^o \cdot S}{K_s + S} \cdot \zeta(\rho) \qquad (1)$$

where μ - measured specific growth rate under different ethanol concentartions; μ_m^o - maximum specific growth rate with no initial ethanol; S-substrate concentration; K_s- Monod saturation constant; $\zeta(\rho)$ - ethanol inhibition function.

The first multiplier of the above equation is simply the Monod equation frequently used in simulating the exponential, retardation, and stationary phases of butch culture as well as chemostat steady state. It applies best to media processing a single growth limiting nutrient, an essential nutrient which runs out completely and stops growth before the concentration of any other nutrient has been reduced to a level which will affect the kinetics. During the course of fermentation process, ethanol accumulates in the broth to such extent that metabolic activities of microorganisms is supressed. Therefore, the presence of ethanol decreases the value of specific growth rate and equation above must be extended to include ethanol inhibition function, $\zeta(\rho)$.

The ethanol inhibition effect typically exhibit different patterns: a) linear kinetics; b) linear kinetics with threshold concentration; c) nonlinear concave up kinetics; and d) nonlinear concave down kinetics (van Uden, 1989).

Several nonlinear ethanol inhibition were found and presented below in the form of the equations by different researchers (Dean, 1964; Aiba et al., 1968; Bazua, 1977; van Uden, 1989) to describe the nonlinear ethanol inhibition:

$$\zeta(\rho) = \frac{K}{K_s + P} \qquad (2)$$

van Uden (1989) has proposed expression for ζ(ρ) in equation above, which is identical with those derivable for noncompetitive inhibition using the Michaelis-Menten equilibrium approach to enzyme kinetics. Such expression, however lack accurate basis when applied to the growth of whole cells.

Equation (3) and Equation (4) below proposed (Aiba et al., 1968; Bazua, 1977) respectively are exponential models from experiments on alcohol fermentation with respiration-deficient mutant of *S. cerevisiae*, where the empirical constant K_p appears to be depend on the method of cultivation (batch or continuous):

$$\zeta(\rho) = e^{-K\rho} \cdot P \qquad (3)$$

$$\zeta(\rho) = 1 - \frac{P}{P_m} \qquad (4)$$

Equation (5) below was suggested (Dean, 1964) to describe the kinetic pattern of product inhibition of the strain *S. cerevisiae*, since there was a nonlinear relation (concavity downward) between μ_i and P. Equation (5) accounts for the influence of ethanol product, however, resulting values were quite different from experimental data.

$$\zeta(\rho) = \left(1 - \frac{P}{P_m}\right)^{f1} \qquad (5)$$

where P - added ethanol concentration; K_P, P_m, and $f1$ - ethanol related constants.

There are several other important parameters that have been identified in the studies for ethanol production from lignocellulosic biomass. Among them arethe net rate of glucose formation and glucose fermentation, respectively by Zhang et al. (2009) as shown in equations below:

$$r_{Gl} = \frac{dGl}{dt} = \left[\frac{K_c \cdot C_b \cdot BG}{K_m \cdot \left(1 + \frac{Gl}{K_{Cg}}\right) + C_b}\right] \cdot 1.053 - \left(\frac{\mu_{Gl}}{Y_{X/Gl}^{max}} + m_{Gl}\right) \cdot X \qquad (6)$$

$$\mu_{Gl} = \left[\frac{X \cdot \mu_{Gl}^{Max} \cdot G_l}{K_{Gl} + G_l + I_1 \cdot X_l}\right] \times \left(1 - \frac{Eth}{Eth_{Gl}^{Max}}\right)^{f1} \qquad (7)$$

where μ_{Gl}^{Max}- maximum specific rate for growth on glucose; Eth_{Gl}^{Max}- maximum ethanol concentration for growth on glucose; $G_l, X_l, and\ Eth$ - concentrations of glucose, xylose and ethanol respectively; C_b, BG, and X - concentrations of cellobiose, β-glucosidase and cell mass; $\mu_{Gl}, Y_{X/Gl}^{max}$, m_{Gl} - specific growth rate, maximum cell mass yield on glucose andmaintenance coefficient on glucose;1.053 - coefficient for water added during cellulose hydrolysis; K_c, K_m, K_{Cg} - cellobiose hydrolysis related constants; K_{Gl}, I_1, f_1 - related constants.

The rate of formation of xylose was described by an approach similar to that used for glucose and could be found elsewhere (Zhang et al., 2009) as in the following equations:

$$r_{Xl} = \frac{dXl}{dt} = -r_{Xn} \times 1.136 - (\frac{\mu_{Xl}}{Y_{X/Xl}^{Max}} + m_{Xl}) \times X \qquad (8)$$

$$\mu_{Xl} = \left[\frac{X \times \mu_{Xl}^{Max} \times (Xl - XlT)}{(K_{Xl} \times X + Xl + I_2 \times Gl)}\right] \times (1 - \frac{Eth}{Eth_{Xl}^{Max}}) \qquad (9)$$

where μ_{Xl}^{Max}, Eth_{Xl}^{Max} - maximum specific growth rate and maximum ethanol concentration for growth on xylose, respectively; I_2, K_{Xl} - related constants; XlT- threshold concentration, which is related to maintenance coefficient m_{Xl} ; $Y_{X/Xl}^{Max}$, μ_{Xl}^{Max}- maximum cell mass yield and maximum specific growth rate; K_{Xl} - Monod saturation constant; 1.136 - coefficient for water added during xylan hydrolysis.

It is been reported (Zhang et al., 2009) that among the microbial growth related constants the maximum specific growth rate on xylose, μ_{Xl}^{max}showed moderate sensitivity, while the

maximum specific growth rate on glucose, μ_{Gl}^{max} showed no sensitivity at all even with a 50% change of the value. Meantime, the ethanol yield from glucose $Y_{Eth/GL}$ exhibits the highest sensitivity, followed by ethanol yield from xylose $Y_{Eth/Xl}$. Ethanol tolerance related constants Eth_{Gl}^{max},Eth_{Xl}^{max}, ζl showed moderate sensitivity. Also, sugar inhibition factors I_1 and I_2 and the Monod constants K_{Xl} and K_{Gl} showed a low sensitivity during the SSCF experimental testing.

At the present time, several biological methods can be used to overcome inhibitory effects of aliphatic acids, furaldehydes or phenolic compounds on yeast fermentation in pretreated lignocellulosic materials. Possible options include detoxification of the pretreated material before fermentation by using available enzyme complexes to mediate enzymatic hydrlosis or use of the natural or targeted genetic engineered bio-reduction capability of the fermenting micro-organism which will detoxify the medium during the fermentation (Parawira and Tekere, 2011). The ability to degrade inhibitors exists in *S. cerevisiae* and other micro-organisms and we only need to exploit or enhance this natural strategy to overcome inhibitors in lignocellulose biomass in some cases through adaptation and genetic engineering. The fermentation can be carried out in a process design such as batch or continues fed-batch that will allow for the natural reduction capability of the micro-organisms to be exploited.

Adaptation (evolutionary engineering) of the fermentation micro-organisms to the lignocellulosic hydrolysate has been also suggested as an alternative detoxification approach (Martin et al., 2007). Although chemical, enzymatic, and microbial detoxification improves the fermentability of hydrolysates, it is desirable to develop adaptive ethanol-producing micro-organisms that require minimal or no detoxification treatment. These adapted organisms not only reduce the detoxification cost, but also avoid loss of fermentable sugars (Martín et al., 2007). Adaptation has been shown to increase the ability of a broad range of yeast strains to grow in

lignocellulosic hydrolysates, resulting in increased fermentation rates and ethanol yields (Parawira and Tekere, 2011). Therefore to conclude, removal of inhibitors is necessary for achieving good fermentation performance even with newly recombinant glucose-xylose utilizing yeasts.

1.7. Process Governing Factors

Evidently, inhibitors play a significant role during the fermentation of the sugars in water-soluble fraction to ethanol. Usually they are composed in one of two categories: process derived inhibitors arising from pretreatment (sugar, lignin from degradation products) or naturally occuring inhibitors liberating from the feedstock and recovered in the water soluble fraction (resin, acids). Inhibitors in both categories play a significant role in the fermentation process. Besides sugar decomposition, lignin is the greatest concern of the process derived inhibitors. Lignin can degrade under certain acidic conditions, primarily by the cleavage of the aryl ether bonds at the α- and β - positions (Lai, 1991). Limited solubilization of lignin via sulphonation has been also reported (Clark et al., 1989) and made this inhibitor an indispensable contributer to inhibiton. The lignin derived phenolics have been shown to inhibit lignocellulosic fermentation, both for the production of ethanol by *S. cerevisiae* and 2,3-butanediol by *Klebsiella pneumoniae* (Nishikawa et al., 1988). Inhibition is related to the disruption of the plasma membrane as well as to the molecular weight of the compound, which affects its permeability (Ando et al., 1986). Removal of these inhibitors could be achieved by extraction with solvents to improve subsequent fermentation step (Clark and Mackie, 1984; Frazer and McCaskey, 1989).

As mentioned early, the naturally occuring inhibitors are mainly of extractives origin. Biologically, many of these extractive components play a defensive role against microbes and

insects in protecting the wood from decay (Haygreen and Bowyer, 1996), and as a result, it's expected to be harmful to fermentation efficiency. However, as for lignin derived phenolics, the concentration of these compounds recovered in the water soluble fraction is often low due to its limited solubility and may not be exceedingly aleatory to subsequent fermentation process.

Among the other important factors that play a considerable role in the fermentation process are pH value and temperature. These factors are well documented and understood in the literature (Lawford et al., 1997; Moniruzzaman et al., 1997; Joachimsthal et al., 1998; Lawford and Rousseau, 1998; Mohagheghi et al., 1998; Teixeira et al., 2000; Mohagheghi et al., 2004; Kim et al., 2008; Zhang, 2008; Lin et al., 2012; Tsuji et al., 2013).

Study of Kim et al. (2008) identified a direct correlation of ethanol production and other factors including pH and temperature in food waste. In their work, response surface methodology (RSM) based on the central composite design (CCD) was used for the optimization of enzymatic saccharification and ethanol production. A combination of factors generating a certain optimum response can be identified. Optimal conditions, particurarly for fermentation, were reported as pH 6.85 and temperature of 35^{o}C. Ethanol yield was obtained as 57.5 g/L under these conditions with a fermentation time of 14 hours. Experimental results were in close agreement with the model prediction and statistical validity. Other comparison study (Zhang, 2008) reported a good performance of strain *Z. mobilis* 8b over strain *S. cerevisiae* RWB222 for SSCF configuration in paper sludge experiments yielding more than 0.38 g/L of ethanol under anaerobic conditions. Table 1.4 outlines some data of this study.

Table 1.4: Selection of strains for paper sludge SSCF

Microorganism	Ethanol Tolerance desired	Good performance near pH 5.5	Good ethanol yield under anaerobic condition	Yield higher than 0.38 with combination of glucose/xylose = 4 under anaerobic condition
E. coli K011	No	No	Yes	Yes
E.coli FBR5	No	No	Yes	Yes
K. oxytoca P2	No	N/A	Yes	Yes
Z. mobilis 8b	Yes	Yes	Yes	Yes
C. shehatae CSIR-Y492	No	No	No	N/A
P. stipitis CSIR-Y633	N/A	Yes	No	No
Saccharomyces sp. 1400	Yes	Yes	No	Yes
S. cerevisiae RWB222	Yes	Yes	Yes	Yes

Source: Zhang, 2008

Results showed paper sludge SSCF by *Z. mobilis* 8b had a much higher ethanol yield at 30^{o}C than 37^{o}C to the better consumption of residual sugar, and higher final glucan and xylan conversion. Yet, paper sludge SSCF by *S. cerevisiae* RWB222 had a higher ethanol yield at 37^{o}C than 30^{o}C, apparently due to enzymatic activity at a higher temperature. It was also concluded that the best ethanol productivity could be achieved at the temperature between 30^{o}C and 37^{o}C with a pH value between 5.0 and 6.0, which support good growth and utilization ability of *Z. mobilis* 8b strain.

The influence factors such as temperature, substrate concentration and pH affecting ethanol fermentation using *S. cerevisiae* BY4742 was demonstrated in Lin et al. (2012) study. Fermentation of sugar by *S. cerevisiae* BY4742 for production of ethanol in a batch experiments was conducted to improve the performance of the fermentation process. Experimental results revealed that the cellsmass increased exponentially at the beginning of incubation,then entered a stationary phase after several days incubation, for different operating temperatures from 20^{o}C to 50^{o}C. Higher temperatures made the exponential growth of the cellsmass shorter. In their study, cell growth and ethanol production declined considerably at 50^{o}C, which showed the inhibition effect on cell growth at higher temperatures. They explained that the high temperature results in changing the transport activity or saturation level of soluble compounds and solvents in the cells, which might increase the accumulation of toxins including ethanol inside the cells.

In the case of substrate concentrations, they found that higher substrate concentration may achieve higher ethanol production, but a longer incubation time was required for higher initial glucose concentration above 80 kg/m^{3}at a temperature of 30^{o}C. However, with glucose concentration of 300 kg/m^3, the ethanol conversion efficiency is decreased considerably (13.7% versus 59.9% for 80 kg/m^3), since the higher substrate and production concentrations may have inhibited the process of ethanol fermentation.

In addition to temperature and substrate concentration, pH is also a key factor that affects on fermentation process for ethanol production. Lin et al. (2012) found that the pH range of 4.0 - 5.0 maybe regarded as the operational limit for the anaerobic ethanol production process. Beyond this range, the formation of by-products, such as acetic and butyric acids may have consumed some of the substrate and reduced the efficiency of fermentation process. Therefore, a robust tolerant yeast is desired to maintain a high ethanol yield with increased substrate loadings

in fermentation phase. Eventually, it will lead to reduction of the amount of enzymes used in pretreatment and enzymatic hydrosis phase, often accounting for up to 40% of the total processing cost (Zhu et al., 2009). Moreover, it greatly affects the downstream cost of fermentation.

CHAPTER 2. THESIS OBJECTIVES AND AUTHORSHIP

2.1. Thesis Objectives

Since the middle of the 70's, significant research and development has brought about numerous research groups for the improvement of lignocellulosic bioconversion to ethanol. However, currently, there is still limited commercialization of a process that can produce the "potential transportation fuel of the future" (Lynd et al., 1996). Most agreed that the present state economy of lignocellulosic ethanol still does not allow commercial production (Banerjee et al., 2010; Zhoa et al., 2012; Baeyens et al., 2015). This is further complicated by the global fluctuations of gasoline prices in recent year. Bioconverison of lignocellulosic biomass into ethanol and other energy value products is regarded as a very complicated process. Nevertheless, clean, renewable energy is always a worthwhile pursuit. There is a great need of continued breakthroughs in advanced technology to achieve this goal.

The current project was initiated at Ryerson University, Toronto, Canadato confirm that various sources of biomass from construction/demolition, organic "green bin" wastes, agricultural and forestry residues can be pre-processed to change the characteristics of biomass. Thereafter, the pretreated biomass can be fermented into alcohols and converted into other useful products such as ethanol, chemicals and gases. This study will lead to the development of an efficient method utilizing non-food-based biomass (e.g. municipal solid waste) to produce ethanol in much more sustainable ways than current practices, which utilize food-based biomass such as corn, sugarcane and wheat more commonly.

The main objective of this research is to investigate the bioconversion process of pretreated source separated organic (SSO) waste by separate hydrolysis and fermentation (SHF) processing approach for ethanol production. The solution is to use "pre-processing" technologies, including the thermal screw press (TSP), cellulose and organic-solvent (ethanol/aceton) based lignocellulosic fractionation (COSLIF) preatretment to fractionate lignocellulosic biomass and prepare it for further fermentation process with bacteria or yeast. Lignocellulosic biomass such as pre-processed SSO waste is particularly attractive since it is widely available, often at negative or low cost and has a great potential for bioconversion in ethanol production.

Specific objectives of the study are: a) verify pretreatment technologies, such as thermo screw press and COSLIF; b) evaluate the performance of the COSLIF pretreatment on SSO feedstock for ethanol production; c) compare the growth and fermentation performances of pretreated SSO waste on ethanol production of two glucose/xylose utilizing strains: *Zymomonas mobilis* 8b and *Saccharamyces cerevisiae* DA2416; and d) interpolate new data of SSO feedstock into an existing kinetic model capable in predicting its behaviour under specified conditions. Figure 2.1 below shows the structure of this thesis which is made up from 3 journal papers published to achieve the specific objectives.

The thermal screw press (TSP) machine is chosen to be used in this study for processing biomass in a new unique way such that the feedstock is exposed to crushing, mixing, homogenizing, granulating, cell decomposition, compacting, heat generating and moisture reduction, all in one step. Compressed products such as peat log, wood chips/briquettes, fertilizer sticks can be extruded and formed into various shapes based on die design. The machine is able to densify on a continuous basis and extrude product in most cases without the need of binders.

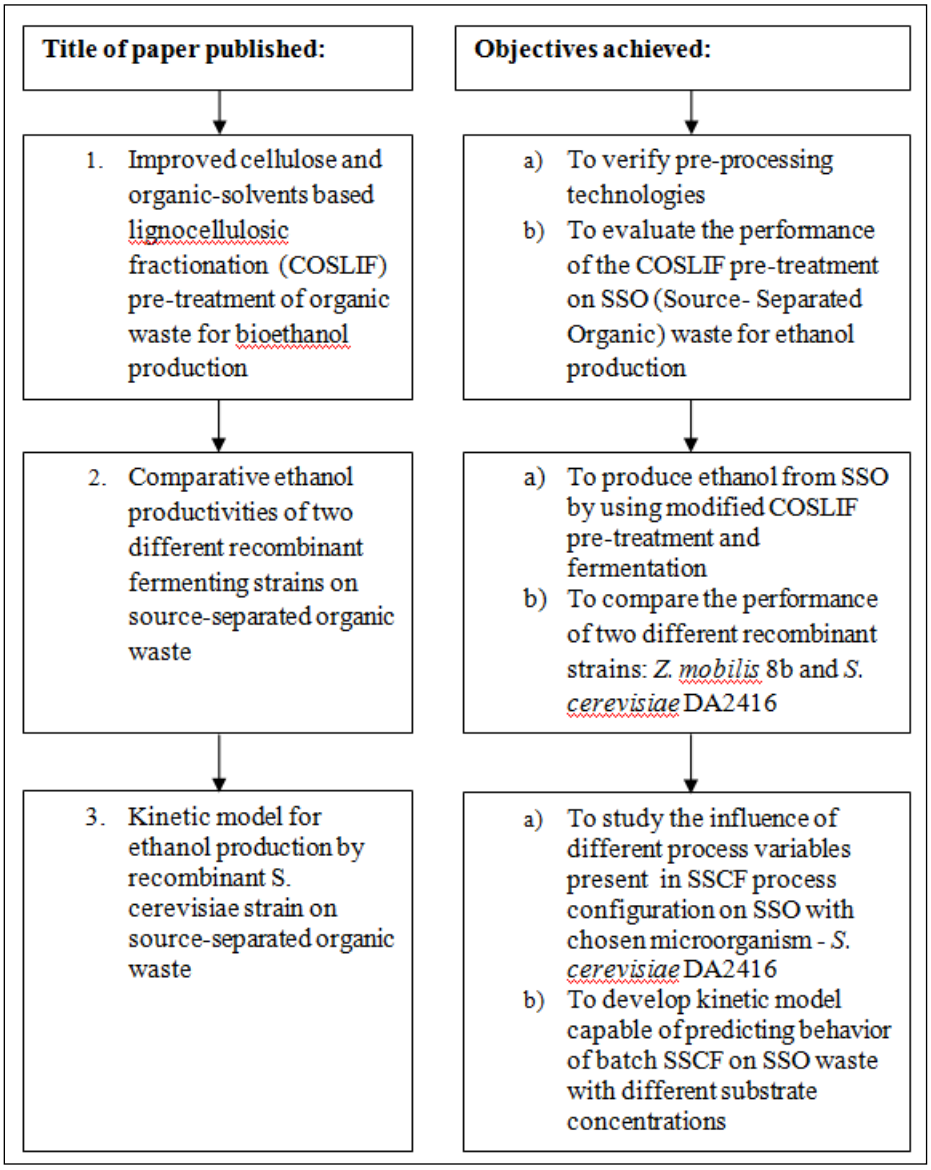


Figure 2.1: Structure and objectives of the study

Among different types of pretreatment technologies, COSLIF pretreatment is adapted in this work due to its impressive glucose yield (approximately 90%), obtained from our preliminary testing (Bekmuradov et al., 2014). COSLIF pretreated SSO substrate will be verified with those

from similar studies and will be further validated with performance of commercially available enzyme complex - Accellerace 1500 in the enzymatic hydrolysis phase.

This study utilized pretreated SSO waste as a model substrate to investigate the SHF configuration with fermentation by two recombinant xylose utilizing strains: *Zymomonas mobilis* 8b and *Saccharomyces cerevisiae* DA2416, which are prominent recombinant strains that utilize xylose and produce ethanol at high yields. The above mentioned strains were chosen for a detailed study of SHF process configuration due to their robustness, ethanol yield, and ethanol tolerance relative to other xylose utilizing recombinant organisms that were tested previously under similar conditions (Zhang, 2008).

This project is a first and potentially the only one of its kind to include process investigation, verification and mathematical modeling on SSO wasteas a feedstock for ethanol production from start to finish. Fermentation and hydrolysis kinetic parameters will be defined experimentally in this study.

Major contributions to the scientific community from this study will include: the understanding of general waste composition from municipal waste streams; cellulose and sugar contents in SSO samples; pretreatment methods that work best for SSO feedstock; comparison of performances on ethanol yields by yeast versus bacterium; and elicitation of significant factors for process control and optimization in the bioconversion process.

It is very timely research with useful application and implications not only in waste management and environmental pollution control, but also in energy sector, food crop, and economics.

2.2. Thesis Outline and Statement of Authorship

2.2.1. Thesis Outline

This thesis is composed of 6 chapters. Chapters 1 and 2 present the introduction and literature review, as well as the main goals for this thesis. Chapters 3 to 5 comprise of three Journal papers describing the main findings of the research program. The performance investigation of COSLIF pretreated SSO is presented in Chapter 3. It describes an improvement on the standard method of COSLIF pretreatment based on lower enzyme loading and using an ethanol washing instead of acetone. Chapter 4 contains a comparison of the growth and fermentation performances of pretreated SSO waste on ethanol productivities of two glucose/xylose utilizing recombinant strains: *Zymomonas mobilis* 8b and *Saccharomyces cerevisiae* DA2416. Chapter 5 describes the mathematical kinetic modeling to accommodate batch simultaneous saccharification and co-fermentation process of the SSO waste by the recombinant strain, *Saccharomyces cerevisiae* DA2416. It includes the model calibration, estimation of parameters, and sensitivity analysis of an existing semi-mechanistic kinetic model as applied to SSO. Chapter 6 presents conclusions and recommendations of future work.

Chapter 1: Introduction

Chapter 2: Thesis Objectives and Authorship

Chapter 3: Improved Cellulose and Organic-Solvent- based Lignocellulosic Fractionation Pretreatment of Organic Waste for Bioethanol Production

This chapter has been published in the American Journal of Engineering Research: Bekmuradov, V., Luk, G., and Luong, R. (2014a). Improved cellulose and organic-solvent-based

lignocellulosic fractionation pretreatment of organic waste for bioethanol production. *American Journal of Engineering Research, 3*(6), 177-185. Available from http://www.ajer.org/papers/v3%286%29/U036177185.pdf

This study investigates the performance of the Cellulose and Organic-Solvent-based Lignocellulosic Fractionation (COSLIF) method for the pretreatment of Source-Separated Organic (SSO) waste. An improvement on the standard method of COSLIF pretreatment was developed based on lower enzyme loading and using an ethanol washing instead of acetone. It was demonstrated that a much higher glucose yield (90% after 72 hours) was possible with this improvement, as compared to the original method, which yielded 70% in the same time frame. Evaluation of the enzymatic hydrolysate obtained from the modified COSLIF pretreatment was further examined by anaerobic fermentation with *Z. mobilis* 8b strain. At 48 hours, ethanol concentration reached to 140 g/L, which is equivalent to 0.48 g of ethanol produced per gram of SSO biomass. This study demonstrated the modified COSLIF pretreatment provides a substantial improvement over the standard method in terms of enzyme savings, glucose formation, and ethanol production.

4: Comparative Ethanol Productivities of two Different Recombinant Fermenting Strains on Source-Separated Organic Waste

This chapter has been published in International Journal of Engineering Research and Applications: Bekmuradov, V., Luk, G., and Luong, R. (2014b). Comparative ethanol productivities of two different recombinant fermenting strains on source separated organic waste. *International Journal of Engineering Research and Applications, 4*(10), 77-82. Available from http://www.ijera.com/pages/v4no10(v5).html

Production of biofuel such as ethanol from lignocellulosic biomass is a beneficial way to meet sustainability and energy security in the future. The main challenge in bioethanol conversion is the high cost of processing, in which enzymatic hydrolysis and fermentation are the major steps. Among the strategies to lower processing costs are utilizing both glucose and xylose sugars present in biomass for conversion. An approach featuring enzymatic hydrolysis and fermentation steps, identified as separate hydrolysis and fermentation (SHF) was used in this work. The proposed solution is to use "pre-processing" technologies, including the thermal screw press (TSP) and cellulose-organic-solvent-based lignocellulosic fractionation (COSLIF) pretreatments. Such treatments were conducted on a widely available feedstock such as source separated organic waste (SSO) to liberate all sugars to be used in the fermentation process. Enzymatic hydrolysis was featured with the addition of commercially available enzyme, Accellerase 1500, to mediate the enzymatic hydrolysis process. On average, the sugar yield from the TSP and COSLIF pretreatments followed by enzymatic hydrolysis was remarkable at 90%. In this work, evaluation of the SSO hydrolysate obtained from COSLIF and enzymatic hydrolysis pretreatments on ethanol yields was compared by fermentation results with two different recombinant strains: *Z. mobilis* 8b and *S. cerevisiae* DA2416. At 48 hours of fermentation, ethanol yield was equivalent to 0.48 g of ethanol produced per gram of SSO biomass by *Z. mobilis* 8b and 0.50 g of ethanol produced per gram of SSO biomass by *S. cerevisiae* DA2416. This study provides important insights for investigation of the source-separated organic (SSO) waste on ethanol production by different strains, and becomes a useful tool to facilitate future process optimization for pilot scale facilities.

5: Kinetic Model for Ethanol Production by Recombinant *S. cerevisiae* Strain onSource-Separated Organic Waste

This paper is currently under review in Computational and Structural Biotechnology Journal.

An existing kinetic model was adapted and modified to accommodate batch simultaneous saccharification and co-fermentation (SSCF) process on source-separated organic (SSO) waste by the recombinant strain, *Saccharomyces cerevisiae* DA2416. The model encompasses enzymatic hydrolysis and fermentation processes with competitive uptake of glucose and xylose in both stages. Enzymatic hydrolysis was featured with the addition of a commercially available enzyme, Accellerase 1500, to mediate the process. Pre-processing technologies, including the thermal screw press (TSP) and cellulose-organic-solvent-based lignocellulosic fractionation (COSLIF) pretreatments, were applied on the SSO waste to liberate fermentable sugars. On average, the sugar yields, mainly in the form of glucose and xylose, from pretreated SSO waste by enzymatic hydrolysis was 90%. The kinetic model was tailored with experimentally-defined SSO parameters to evaluate the sugar and ethanol yields from SSO waste, and was found to predict ethanol production rate accurately with diminutive variance from experiments. Experimental results demonstrated that *S. cerevisiae* DA2416 produced more than 150 g/L ethanol, with ethanol yield of 0.50 g of ethanol/g potential sugar fed, in less than 5 days with 96% cellulose conversion. It was confirmed in this work that cellulose adsorption capacities along with hydrolysis rate constant have a high impact on sugar and ethanol formation.

This study provides important insights for investigation on the use of SSO waste for ethanol production by *S. cerevisiae* DA2416 and the model is proven to be a useful tool to facilitate future process optimization for pilot scale facilities.

Chapter 6: Conclusions and Future Work Recommendations

2.2.2. Statement of Authorship

Chapter 1: Introduction

Valeriy Bekmuradov wrote initial draft with input from Prof. Grace Luk.

Chapter 3: Improved Cellulose and Organic-Solvent-based Lignocellulosic Fractionation Pretreatment of Organic Waste for Bioethanol Production

Valeriy Bekmuradov, Grace Luk and Robin Luong.

Valeriy designed the experiments of this chapter with inputs from Prof. Grace Luk and Robin Luong.

Valeriy performed all experimental work and data analysis. Writing was completed with inputs from co-authors.

Published in 2014: *American Journal of Engineering Research*, *3*(6), 177-185.

Chapter 4: Comparative Ethanol Productivities of two Different Recombinant Fermenting Strains on Source-Separated Organic Waste

Valeriy Bekmuradov, Grace Luk and Robin Luong.

Valeriy designed the experiments of this chapter with inputs from Prof. Grace Luk and Robin Luong.

Valeriy performed all experimental work and data analysis. Writing was completed with inputs from co-authors.

Published in 2014: *International Journal of Engineering Research and Applications, 4*(10), 77-82.

Chapter 5: Kinetic Model for Ethanol Production by Recombinant *S. cerevisiae* Strain on Source-Separated Organic waste

Valeriy Bekmuradov and Grace Luk.

Valeriy designed the experiments and perform the mathematical modeling of this chapter with input from Prof. Grace Luk.

Valeriy performed all experimental work and data analysis. Writing was completed with inputs from Prof. Grace Luk.

Submitted to: *Computational and Structural Biotechnology Journal*

CHAPTER 3. IMPROVED CELLULOSE AND ORGANIC-SOLVENT-BASED LIGNOCELLULOSIC FRACTIONATION PRETREATMENT OF ORGANIC WASTE FOR BIOETHANOL PRODUCTION

Abstract

This study investigates the performance of the Cellulose and Organic-Solvent-based Lignocellulosic Fractionation (COSLIF) method for the pretreatment of Source-Separated Organic (SSO) waste. An improvement on the standard method of COSLIF pretreatment was developed based on lower enzyme loading and using an ethanol washing instead of acetone. It was demonstrated that a much higher glucose yield (90% after 72 hours) was possible with this improvement, as compared to the original method, which yielded 70% in the same time frame. Evaluation of the enzymatic hydrolysate obtained from the modified COSLIF pretreatment was further examined by anaerobic fermentation with *Zymomonas mobilis* 8b strain. At 48 hours, ethanol concentration reached to 140 g/L, which is equivalent to 0.48 g of ethanol produced per gram of SSO biomass.

This study demonstrated that the modified COSLIF pretreatment provides a substantial improvement over the standard method in terms of enzyme savings, glucose formation, and ethanol production.

3.1. Introduction

Pretreatment is considered one of the most expensive processing steps in the bioconversion of lignocellulosic biomass, often accounting for up to 40% of the total processing cost (Zhu et al., 2009). In addition, it greatly affects the downstream cost of operations such as

enzymatic hydrolysis and fermentation. Additional costs resulting from inefficient pretreatment include detoxification, limited enzymatic hydrolysis rate, high enzyme loading, low product concentration, and complicated product purification. Therefore, pretreatment can be seen as a key step in limiting the feasibility of bioconversion. Pretreatment, together with enzymatic hydrolysis, is the central task of the entire bioethanol production process (Zhu et al., 2009). Evidently, all the lignocellulosic pretreatment processes experience sugar degradation and inhibitor formation. The shortfalls of the current leading lignocellulosic pretreatments can be mainly attributed to: 1) inefficiency in breaking up the orderly hydrogen bonds in crystalline cellulose, resulting in slow hydrolysis rates and low cellulose digestibility, which compromises the overall sugar yields, and 2) the presence of lignin and hemicellulose on the surface of cellulose, which is commonly thought to have the effect of restricting the accessibility of enzymes to the biomass (Zhang et al., 2007).

Cellulose and Organic-Solvent-based Lignocellulosic Fractionation (COSLIF) is a promising technology, recently developed to overcome these problems. The COSLIF pretreatment is a technology that can effectively fractionate lignocelluloses into amorphous cellulose, lignin, hemicelluloses, and acetic acid (Zhang et al., 2007; Rollin et al., 2011). This technology has been applied successfully to a broad range of substrates from agricultural to industrial waste, with inclusion of organics such as: food, paper, cardboard, plastics and yard wastes (Zhang et al., 2007; Sathitsuksanoh et al., 2009; Zhu et al., 2009; Ge et al., 2011; Sathitsuksanoh et al., 2011). The COSLIF technology has many advantages over traditional lignocellulosic pretreatments, most notably the following: modest treatment conditions at 50°C and atmospheric pressure; minimized degradation of sugars; no inhibitor formation; co-utilization of different sugars increasing potential output; high sugar yields; fast hydrolysis rates;

efficient solvent recycling; low usage of enzymes; and low energy consumption (Zhang et al., 2007).

The objective of this paper is to evaluate the performance of two COSLIF pretreatment methods: standard and modified, on an innovative feedstock for ethanol production, namely, source-separated organic (SSO) waste. Due to its potential for high energy content and environmental implications, SSO has been proposed as a suitable feedstock for bioethanol production (Mirzajani, 2009). It was demonstrated that the overall process of lignocellulose fractionation with the use of cellulose solvent (phosphoric acid) and organic solvent (acetone/ethanol) as pretreatment reagents is effective in hydrolyzing the sugar content of the waste (Zhang et al., 2007). In order to successfully deal with the causes of the SSO recalcitrance - breaking up orderly hydrogen bonds in the crystalline cellulose chain and removing lignin and hemicelluloses from the surface of cellulose, a standard COSLIF process was modified by using ethanol washing solvent instead of acetone and lowering enzyme loading. It allowed increase in the concentration of glucose released after enzymatic hydrolysis and to achieve the highest ethanol yield in the fermentation step. The enzymatic hydrolysis performances of the original and modified COSLIF pretreatment methods were investigated and compared in terms of their glucose yield. A scanning electron microscopy (SEM) was used to examine the supra-molecular structures of COSLIF-pretreated SSO samples for qualitative comparison.

3.2. Materials and Methods

The SSO waste utilized in this work was initially pre-processed mechanically, under high temperature (of approximately120^{o}C) and pressure (over 50 bars) with a thermal screw press to

form a semi- dry stable biomass. The thermal screw press (TSP) machine by the Aufbereitungs Technologie and System (ATS) AG, Switzerland, is a heavily built piece of processing machinery that fits into the dimensions of five meters by two meters by fifteen meters and weighs between six to seven tones, depending on the model. The machine has a twin parallel extruding screws, which run the length of the machine and pass through one to three processing chambers. These screws carry feedstock through a thermal friction processing technique created through the adjusting the friction plates that are located between the chambers. TSP is powered either by electric or diesel motors and can process biomass and other waste materials in a completely the way, such that the feedstock would grind, compress and create an effecton organic materials and carry out this function in one step. During the operation of TSP, heat is generated through friction caused by the forward pressure and turning action of the screws. Normal operating temperature run between 105 and 125 degree C and pressure varies from 50 to 290 bar depending on setting needs and feedstock origin. The flexible operating principles of this machine offers great potential for processing different lignocellulosic feedstocks (Vartek Waste Management Ltd, 2005).

SSO samples were prepared as a heterogeneous substrate by blending approximately 80% organic waste with 20% woodchips from construction/demolition waste before pre-processing (Vartek Waste Management Ltd, 2005). Optimum Waste Recycling Systems, Toronto, Canada, supplied the biomass feedstock used in this work. The general flowchart of the experimental investigation is shown in Figure 3.1.

It started with the SSO waste fed to thermo-screw press and to make it homogenous. After this, the SSO samples underwent lignocellulosic fractionation with the use of a cellulose solvent (85% phosphoric acid) and an organic solvent (either acetone or ethanol). The next step

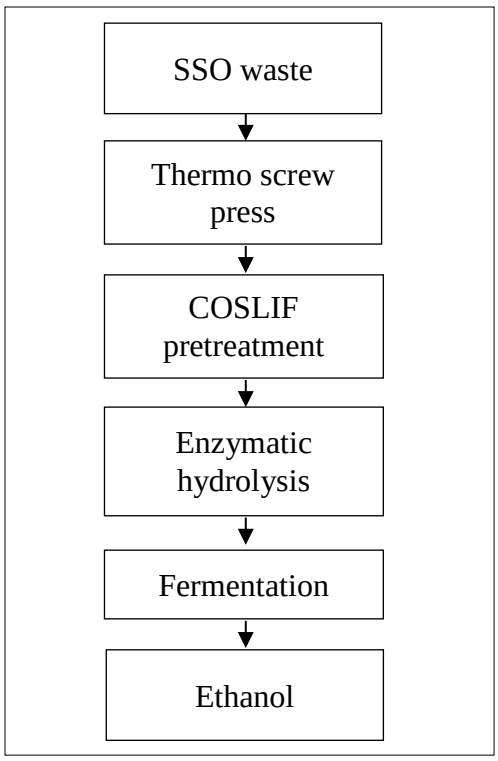


Figure 3.1: Experimental flowchart.

in the flowchart above is enzymatic hydrolysis with the addition of commercially available enzyme, Accellerase 1500, to mediate enzymatic hydrolysis process and release all fermentable sugars available for further fermentation. Accellerase 1500 is Genencor's one of the innovative enzyme products, with a significant step forward towards more cost effective, commercial scale production developed for second generation of biorefineries. It has been proven that Accellerase 1500 could successfully hydrolyze a wide range of lignocellulosic feedstocks (Retka, 2009). Accellerase 1500 enzyme used in this research was supplied by Genencore Inc., a Denisco Division, Rochester, New York, USA, as well as by the Sigma Aldrich Corp., USA.

Prior to testing, the SSO samples were oven-dried at 45^{o}C -50^{o}C for 72 hours following recommended practice (Hames et al., 2008). Five grams of dry lignocelluloses was placed in a

250 mL centrifuge bottle and then mixed with 40 mL of 85% concentrated phosphoric acid using a glass rod (see Appendix D for details on experimental procedures).

The solid/ liquid slurry was placed in a benchtop shaking incubator at 150 rpm and 50^{o}C ± 0.2^{o}C for 2 hours. One hundred mL of ethanol was then added and mixed well. After centrifugation at 7000 rpm at room temperature for 15 minutes, the supernatant was decanted. The solid pellet was then re-suspended by 150 mL of ethanol and centrifuged. The supernatant again was decanted. Next, the solid pellet was re-suspended by 150 mL of distilled water and centrifuge two additional times (Zhang et al., 2007; Dowe and McMillan, 2008).

Enzymatic hydrolysis experiments were conducted next in sequence in the chosen SHF approach in a benchtop shaking incubator. The separate hydrolysis and fermentation (SHF) approach was used in this study to avoid interference of samplings. The procedure for enzymatic cellulose hydrolysis was adopted from a procedure developed by the National Renewable Energy Laboratory and described in (Ehsanipour, 2010; Brown and Torget, 1996). After thawing, the treated solid pellet containing amorphous cellulose was neutralized to pH 4.8-5.0 by NH4OH. Upon diluting to 20 g glucan/L based on the 27% glucose content (Ehsanipour, 2010), the sample was then brought to 50^{o}C before adding 30 FPU/ g glucan or 60 FPU/g glucan of Accellerase 1500. The incubator was set at 250 rpm to keep solids in constant suspension with the temperature of 50^{o}C for 72 hours. Sampling was carried out at 0, 12, 24, 48 and 72 hours and glucose yield was measured.

Following enzymatic hydrolysis, batch soluble sugar fermentation was carried out to determine the ethanol yields. The *Z. mobilis* 8b recombinant strain was chosen for its capability to ferment glucose and to produce ethanol at high yields (Mohagheghi, 2004) and was donated by the National Renewable Energy Laboratory, Golden, Colorado, USA. Soluble sugars batch

fermentation was performed in 250-mL serum bottles with 100-mL working volume and purged with nitrogen before being autoclaved. Temperature was maintained at 30^{o}C-37^{o}C and pH was controlled at 5.0-6.0 by 1M potassium hydroxide (KOH) as suggested by previous studies (Zhang et al., 2009). Each batch sugar fermentation process was carried out in triplicates on the pretreated biomass for both the standard and modified COSLIF methods.

Concentrations of glucose in hydrolysates from the COSLIF pretreated biomass and ethanol from in fermentation broths were analyzed by high performance liquid chromatography (HPLC), Bio-Rad HPX-87P column quipped with the appropriate guard column. All concentrations were reported as per liter volume basis. Percent theoretical ethanol yield was calculated (Dowe and McMillan, 2008):

$$\% \text{ Theoretical ethanol yield} = \frac{[EtOH]f - [EtOH]i}{0.51 \cdot (f \cdot [Biomass] \cdot 1.111) \cdot 100}$$

where $[EtOH]_f$ - ethanol concentration at the end of fermentation, (g/L); [EtOH]i - ethanol concentration at the beginning of fermentation, (g/L); [Biomass] - the dry biomass concentration at the beginning of fermentation, (g/L); f - the cellulose fraction of dry biomass (g/g); 0.51 - the conversion factor for glucose to ethanol; 1.111 - conversion factor for cellulose to equivalent glucose.

Supra-molecular structures of the intact and pretreated SSO samples were examined by scanning electron microscope, as described elsewhere (Zhang et al., 2006; Selig et al., 2007). A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a

rectangular pattern of image, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Samples can be observed in high and low vacuum, and in wet conditions. A SEM was kindly provided by the Ryerson University Analytical Center, Toronto, Canada.

3.3. Results and Discussion

A detailed quantitative assessment on the composition of SSO waste was carried out (Ehsanipour, 2010) and adopted for further investigation in this study. The SSO samples, contained 20% woodchips, were already pretreated by the thermal screw machine. The woodchips were typically Douglas fir wood waste originated from home construction furniture, flooring, cabinet, and doors. All sharp foreign matter such as metal needles, plastic and rubber wastes, and broken glasses were collected and removed, as much as it was possible. The dried SSO biomass was sent to MBI International, the Michigan State University Foundation, for grinding and determination of polymeric sugars content. The results are summarized in Table 3.1. It turned out that those essential polymeric sugars made up 41.3% in oven dried SSO samples, including: 27% glucan, 5.4% xylan, 5.7% mannan, 1.2% arabinan, and 1.2% of galactan, which were a good starting point for enzymatic hydrolysis followed by fermentation. It was found that the SSO samples were acidic (pH of 5.0-5.5) and had the highest content of the food waste, just about 80% of total waste of samples. Comparison between pretreated and non-treated SSO validated the high recalcitrant nature of lignocellulosic fraction of biomass as suggested by Zhang et al. (2007), and which was in agreement with other works (Zhang et al., 2009; Zhu et al.,2009; Rollin et al., 2011).

Table 3.1: Compositional analysis of source-separated organic samples

Parameters	Value
A. Physical Properties	
Biomass as received	
pH	5 @ 25°C
Total Solids (TS)	33.14%
Moisture content	66.86%
VOC per dry mass	28.00%
Ash per dry mass	5.14%
Oven-dried and homogenized biomass	
pH	5.5 @ 25°C
Moisture content	6.60%
TS	93.40%
VOC	83.40%
Ash	16.60%
B. Structural Carbohydrate and Lignin	
(per oven-dried and homogenized biomass)	
Starch	NS
Free Sugar	NS
Glucan	26.80%
Xylan	5.40%
Arabinan	1.20%
Mannan	5.70%
Galactan	2.20%
Total sugars	41.26%
Acid Insoluble Lignin (AIL)	25.40%
Acid Soluble Lignin (ASL)	1.20%
Total Lignin	26.60%
Acetic, Lactic and Formic acids	NS
C. Others	
Total Kjehldahl Nitrogen (TKN)	5450 µg/g
Extractives	11.00%
Digestibility	12.70%
Biodegradability	82.00%

Source: Ehsanipour, 2010

NS - not significant

3.3.1. Glucose Yield

Results obtained from COSLIF washing (shown in Figure 3.2) with concentrated phosphoric acid and acetone reagent generated a significant glucose yield of about 70%, in the first few trials.

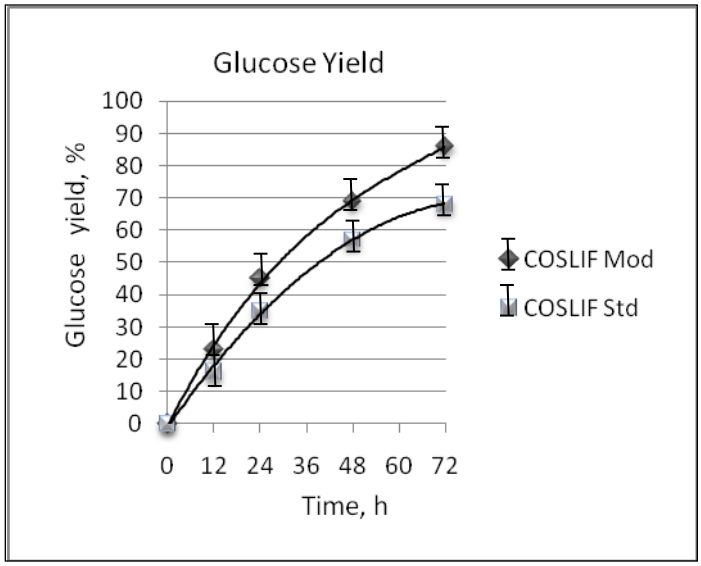


Figure 3.2: Glucose yields of standard and modified COSLIF pretreatment performed at 50°C for 72 hours

However, acetone is a more toxic reagent and it is less safe to use than ethanol. The cost of using acetone is higher than that of ethanol and during the recovery of the reaction's by-products more energy is consumed when acetone is used as the reagent. In addition, pretreatment with acetone must be performed under extremely stringent and efficient conditions due to the volatility of acetone. Ethanol, on the other hand, is less corrosive and can be easily recovered by distillation under milder conditions. Therefore, after extensive trials and investigations, some changes were made to further improve the efficiency of COSLIF pretreatment to obtain a higher

glucose yield. The major change made to the original standard method of COSLIF pretreatment was to omit acetone altogether and use 95% (v/v) ethanol as the organic solvent instead. Another was changing enzyme loading from 60FPU to 30FPU. As a result of these changes, the glucose yield increased to approximately 90% (Figure 3.2). Additionally, only 50% of the original volume of ethanol was needed to replace the acetone.

3.3.2. Enzymatic Hydrolysis

Figure 3.3 shows the glucose digestibility profiles over a course of 72 hours for the SSO samples treated by the standard and modified COSLIF methods as well as non-treated samples.

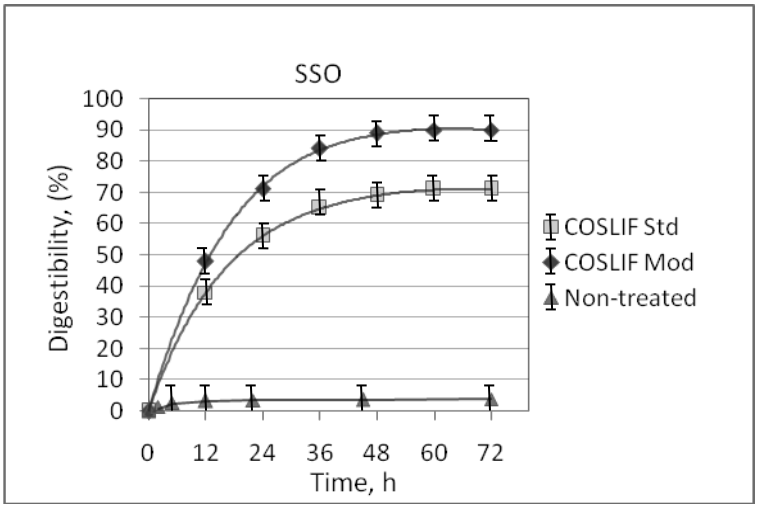


Figure 3.3: Time trend of glucose digestibility from the non-treated to standard and modified COSLIF pretreated samples

High glucan digestibility of the pretreated SSO was accredited to drastic changes in the supramolecular structure of the biomass before and after the COSLIF pretreatment, observed by

the SEM in this study. Typical COSLIF pretreatment conditions were used, namely 50^{o}C and atmospheric pressure with a pretreatment time from 30 to 60 minutes, depending on the type of feedstock. Although diverse feedstocks showed great variations in enzymatic digestibility, suggesting that their different recalcitrant structures confer variable resistance to enzymes, the use of concentrated phosphoric acid at 50^{o}C can efficiently dissolve them so to erase their inherent structure difference and result in an amorphous biomass with similar high-accessibility (Rollin et al., 2011; Sathitsuksanoh et al., 2011). As a result, COSLIF-pretreated biomass feedstock exhibited similar enzymatic glucan digestibility regardless of their sources (Sathitsuksanoh et al., 2011). When concentrated phosphoric acid was used as the cellulose solvent, it should be used at 50^{o}C or lower to avoid extensive hydrolysis of polymeric carbohydrates and sugar degradation.

The enzymatic glucose digestibility for pretreated COSLIF samples was calculated as described by Zhang et al. (2007). With high enzyme loading, 60FPU and acetone washing, the glucose digestibility of the pretreated standard COSLIF sample was approximately 70% as presented in Figure 3.3 above. With a lower enzyme loading, 30FPU and ethanol washing, it reached 90% digestibility after 36 hours. This suggests that by removing hemicelluloses and lignin barriers, there was an increase in accessibility to the cellulose change by the cellulobiose, while also reducing the competitive inhibition of xylan to endo-glucanase. Data from this study on the hydrolysis rates and digestibility were comparable to the range (90%-95%) cited in other scientific papers (Mosier et al., 2005; Wyman et al., 2005).

3.3.3. Fermentation

Fermentation is the final step in evaluating the overall process of cellulosic ethanol production. The effectiveness of the enzymatic hydrolysis was gauged by assessing the potential inhibitory factors and effects of fermentation. These results can be found in the following section. A genomic DNA-integrated glucose and xylose co-fermenting strain, *Z. mobilis* 8b recombinant strain was used due to its ability to ferment glucose and xylose to produce ethanol at high yields (Mohagheghi et al., 2004). The microbe was developed and evaluated by the NREL on a broad range of agricultural biomass and can convert sugars to ethanol more rapidly as compared to other species.

Besides the major changes during the COSLIF pretreatment process, some minor improvements in the fermentation procedure were also made and they undoubtedly affected overall efficiency of the final ethanol output. These improvements were as follows: a serum bottle with a crimp top was used instead of an Erlenmeyer flask with stopper for better air-tight seal; a flushing serum bottle with nitrogen was used to maintain anaerobic conditions prior to fermentation; a direct transfer technique was exploited to move concentrated *Z. mobilis* 8b cells from an inoculums tube to a serum bottle; and a growth curve was developed for the *Z. mobilis* 8b strain prior to fermentation tests which was important in order to identify the OD (optical density) range in the exponential phase of a curve. The OD values in the exponential phase were vital in determining the time to harvest the cells to start the fermentation process. There were two protocols that could be employed for harvesting the cells to start the fermentation process: 1) use of a direct transfer (10%) to the main fermentation bottle or 2) use of concentrated cells by centrifuging in a centrifuge tube and then re-suspending the cells in a hydrolysate before transferring it back into the fermentation bottle. The second protocol was chosen because the

inoculated seed media contained not only cells but also a large amount of glucose sugar which would be transferred into the fermentation bottle. Unless distilled deionized water (DDW) blank was created, this would result in false and inaccurate HPLC readings of glucose and ethanol concentrations.

The high ethanol yield shown in Figure 3.4 indicated that very little inhibitors were present in the hydrolysates that were pretreated by the modified COSLIF method. Depending on feedstock and process, the actual yield could be anywhere from 60% to 100% of the theoretical yield. Achieving a high yield may be costly compared to lower yield processes that are often more cost effective.

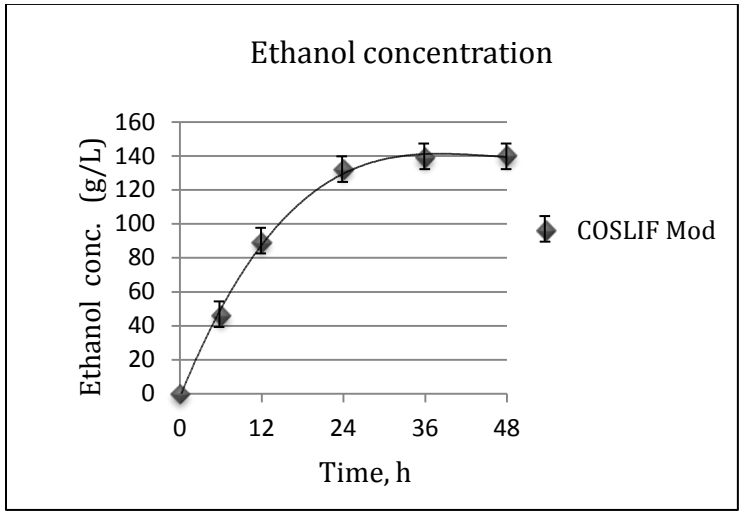


Figure 3.4: Ethanol concentration from modified COSLIF pretreated samples

The ethanol concentration rate was calculated on the basis of sugars consumed as described by South et al. (1995), and it yielded in 132.1 g/L for the pretreated samples by the modified COSLIF method after 24 hours. At 48 hours, the ethanol concentration reached 140 g/L, which is equivalent to 0.48 g ethanol/g biomass or 94% of the theoretical ethanol yield. As

per this work, percent theoretical ethanol yield was calculated accordingly (Dowe and McMillan, 2008). Although the ethanol concentration for some samples seemed to be fluctuating from time to time, over 90% ethanol yield can be attributed to the high accessibility of the pretreated cellulosic materials and low presence of lignin.

3.3.4. Comparison with Constructed Sugar Model

In a further series of experimental evaluations, enzymatic hydrolysate obtained from both COSLIF pretreatments by batch culture fermentation with *Z. mobilis* 8b strain were compared with constructed sugar model (glucose/xylose ratio as 5:1) in SSO substrate. In a constructed model, after 24 hours, 100% of glucose and 40% of xylose were consumed. While in the enzymatic hydrolysate, pretreated by COSLIF with ethanol washing reagent, the fermentation also advanced rapidly and 90% glucose and 40% xylose were also consumed, in the enzymatic hydrolysate, pretreated by COSLIF with acetone washing reagent, the fermentation advanced slowly and 45% of glucose remained unused in the same period of time. Low bacterial activity in the fermentation process of SSO hydrolysates may be attributed to many factors including: longer lag phase for *Z. mobilis* 8b strain as the adaptation time to growth condition, low growth rate on SSO hydrolysates, unavoidable contamination during sample preparations, lack of nutrients, and presence of inhibitors.

3.3.5. Qualitative Analysis

As per qualitative comparison, SEM images of oven-dried SSO substrate before and after pretreatment were conducted in collaboration (Ehsanipour, 2010) and provided in Figure

3.5.These images show the appearance of SSO before grinding – 1-1, after grinding – 2-1, and after COSLIF pretreatment – 3-1. Each pretreatment (physical and chemical) process changed the structure of the SSO biomass. Before the pretreatment, the plant cell wall structures of the SSO and cellulose fibers were clearly identified. The SEM images from 1-1 and 2-1present changes in particle size.

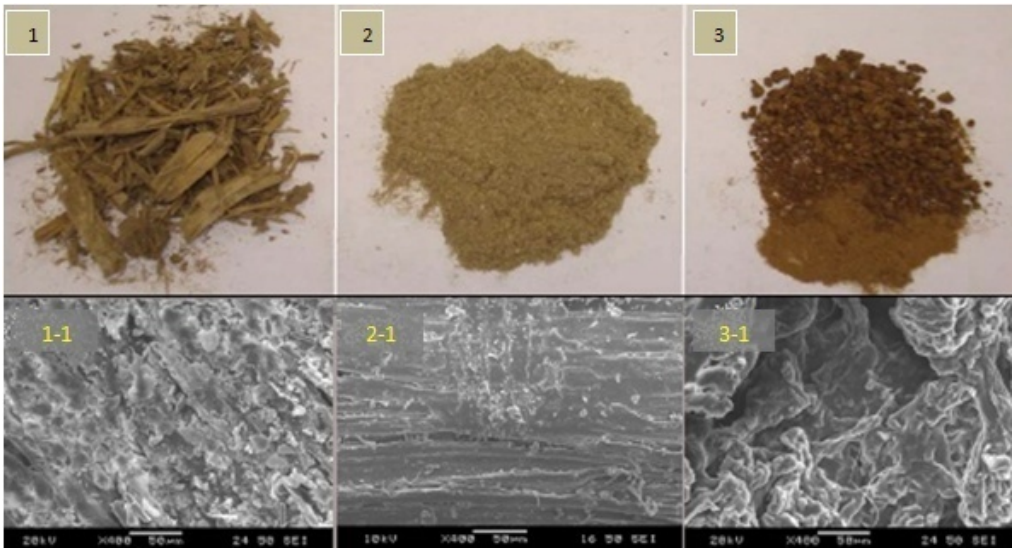


Figure 3.5: Scanning electron microscopy images of source-separated organic waste

Source: Ehsanipour (2010)

The image from 3-1 shows all fibrous structures completely disrupted after pretreatment, indicating phosphoric acid and ethanol washing not only disrupted all linkages among cellulose, hemicelluloses and lignin, but also disrupted the orderly hydrogen bonds among glucose chains. These qualitative images are consistent with the images from similar studies (Zhang et al., 2006; Zhu et al., 2009).

3.4. Conclusions

The SSO waste samples utilized in this research were pre-processed by the thermal screw press (TSP) and further used as a substrate for all enzymatic hydrolysis and fermentation processes.SSO has an excellent potential to be utilized as a feedstock for ethanol production due to its high fermentable sugar content, 40% - 42%. On the other hand, SSO has high lignin content, which is slightly above 26% per kg of dried feedstock emphasizes need for pretreatment.

COSLIF pretreatments were applied for cellulose extraction. Results indicate the percent glucose conversion was considerable for the modified COSLIF method with significant glucose yield, just above 90%. This study also demonstrated and confirmed that the COSLIF pretreatment can be carried out on this innovative type of biomass with a relatively high percentage of glucose and ethanol yields, when certain modifications are made to the process: a) the efficiency of using modified COSLIF pretreatment was improved by 20% using ethanol instead of acetone; b) using serum bottle with crimp top in fermentation process instead Erlenmeyer flask with stopper for air tight seal; c) using concentrated cells during fermentation phase instead of direct transfer mode to avoid false HPLC reading; and d) decrease enzyme loading from 60FPU to 30FPU for cost reduction. However, there are still some aspects of the process that need further investigation. For example, biomass size reduction by milling or grinding is energy intensive and costly which will affect the total cost of ethanol production. The extrusion process alone could disrupt the lignocellulosic structure, which would enable enzymes to gain access and attack the carbohydrates (Alvira et al., 2010). Detailed investigation on ethanol concentration and yield is still required. It was hypothesized that the large variations of ethanol concentration in this study were caused by interference of samplings. However, this has yet to be proven.

For supporting materials on this chapter please refer to Appendix A “Glucose and ethanol yields calculations”.

CHAPTER 4. COMPARATIVE ETHANOL PRODUCTIVITIES OF TWO DIFFERENT RECOMBINANT FERMENTING STRAINS ON SOURCE-SEPARATED ORGANIC WASTE

Abstract

Production of biofuel such as ethanol from lignocellulosic biomass is a beneficial way to meet sustainability and energy security in the future. The main challenge in bioethanol conversion is the high cost of processing, in which enzymatic hydrolysis and fermentation are the major steps. An approach featuring enzymatic hydrolysis and fermentation steps, identified as separate hydrolysis and fermentation (SHF) is proposed to eliminate this problem. The solution is to use "pre-processing" technologies, including the thermal screw press (TSP) and cellulose-organic-solvent-based lignocellulose fractionation (COSLIF) pretreatments. Such treatments are conducted on ground-breaking feedstock such as source separated organic waste (SSO) to liberate all sugars to be used in the fermentation process. TSP and COSLIF pretreatments followed by enzymatic hydrolysis were applied on the SSO to unlock fermentable sugars (glucose and xylose) for ethanol production. Enzymatic hydrolysis was featured with addition of commercial available enzyme, Accellerase 1500, to mediate enzymatic hydrolysis process. On average, the sugar yield from the TSP and COSLIF pretreatments followed by enzymatic hydrolysis was remarkable at 90%. In this work, evaluation of the SSO hydrolysate obtained from COSLIF and enzymatic hydrolysis pretreaments on ethanol yields was compared by fermentation results with two different recombinant strains: *Zymomonas mobilis* 8b and *Saccharomyces cerevisiae* DA2416. At 48 hours of fermentation, ethanol yield was equivalent to 0.48 g of ethanol produced per gram of SSO biomass by *Z. mobilis* 8b and 0.50 g of ethanol produced per gram of SSO biomass by *S. cerevisiae* DA2416.

This study provides important insights for investigation of the source-separated organic (SSO) waste on ethanol production by different strains and becomes a useful tool to facilitate future process optimization for pilot scale facilities.

4.1. Introduction

Ethanol production from lignocellulosic biomass has a potential to be a viable replacement or supplement for fossil fuel, but the current cost of conversion is a major bottleneck for commercial application (U.S. Department of Energy, 2006). The price for ethanol remains as high as $2.75 per gallon motivating further research (Collins, 2007). By contrast the average price for regular, unleaded gasoline in the USA is currently hovering around $3.9 per gallon with expectation for it to rise even more (U.S. Department of Energy, 2014). It became apparent that in efforts to reduce the production costs of ethanol, improvements in several areas of biofuel production including feedstock, price design, and enzymes are required. At the present time, there are at least two methods of ethanol production from lignocellulose that are in advanced phases of development: enzymatic hydrolysis and biomass fermentation. Neither process generates toxic emissions while producing the end product, ethanol. The technology is relatively new and exists in pilot configurations where testing is ongoing. While today ethanol is mostly produced from starch contained in grains such as corn, sugarcane and grain sorghum, it can also be produced from cellulose which is mainly present in non-food products. Currently, lignocellulosic feedstock is the most abundant biomass, which has attracted considerable attention and is often a major or the sole component of different waste streams from various

industries including agriculture, forestry, and municipalities' wastes (Taherzadeh and Karimi, 2008).

Today's bioethanol technology has offered sustainable approaches to the problem with municipal solid waste (MSW) by focusing on utilization of organic fraction of solid waste and agriculture residue in order to reduce wastes and avoid conflicts between human food and industrial use of crops. Organic fraction of solid waste has given a new perspective to the industry by defining an innovative system for converting trash into bioethanol reducing the amount of waste piling up in landfills, while displacing a large fraction of the fossil fuels to power vehicles. Biomass such as processed source separated organic (SSO) waste is particularly attractive in one context since it is widely available at a negative cost and has many other environmental benefits. It provides a good alternative fuel in terms of green-house gas emissions, reduction of farmland's depletion, and diminutive of generated waste.

Ethanol yield and productivity are the key parameters in the production of biofuel from biomass and wastes. The fermentation of xylose-to-ethanol is important in biomass-to-ethanol process since it can increase ethanol yield up to 50% (Hinman et al., 1989). Several strains have been engineered to ferment xylose to ethanol (Hahn-Hagerdal et al., 1993; McMillan, 1994; Mohagheghi et al., 2004). Among them are *Zymomonas mobilis*, *Saccharamyces cerevisiae*, and *Pitchia stipulus*. The first two abovementioned strains met the selection criteria which were based on several fermentation characteristics considered to be essential for biomass-to-ethanol conversion (Picataggio et al., 1994; Zhang et al., 1995).

The purpose of this study was a comparison of the growth and fermentation performances of pretreated source-separated organic (SSO) waste on ethanol productivities of two glucose/xylose utilizing recombinant strains: *Z. mobilis* 8b and *S. cerevisiae* DA2416. The

feasibility of the SSO as a potential feedstock for ethanol production has been widely demonstrated (Mirzajani, 2009; Percy, 2009; Ehsanipour, 2010; Faye, 2010; Luong, 2012; Bekmuradov et al., 2014a). Before pretreatment, a compositional characterization of pre-processed SSO samples collected at the City of Toronto, Ontario, Canada for a ten-month period was carried out (Mirzajani, 2009).

4.2. Materials and Methods

The SSO waste samples intended in this research were pre-processed mechanically under high temperature and pressure by the thermal screw press (TSP) and then used as a substrate for all enzymatic hydrolysis and fermentation processes. Moreover, the SSO waste samples were made as a heterogeneous substrate of demolished construction waste blended with approximately 20% of woodchips and 80% organic "green bin" waste and pre-processed accordingly (Vartek Waste Management Ltd, 2005). Prior to testing the SSO waste was oven dried at 45^{o}C-50^{o}C for 48 hours.

The next step encompassed lignocellulosic fractionation by cellulose-solvent (phosphoric acid) and organic-solvent (ethanol). Five grams of dry lignocelluloses was placed in a 250 mL centrifuge bottle and then mixed with 40 mL of 85% concentrated phosphoric acid using a glass rod. The solid/ liquid slurry was placed in a benchtop shaking incubator at 150 rpm and 50^{o}C ± 0.2^{o}C for 2 hours. One hundred mL of ethanol was then added and mixed well. After centrifugation at 7000 rpm at room temperature for 15 minutes, the supernatant was decanted. The solid pellet was then re-suspended by 150 mL of ethanol and centrifuged. The supernatant

again was decanted. Next, the solid pellet was re-suspended by 200 mL of distilled water and centrifuge two times and stored in a freezer for a short period of time (Rollin et al., 2011).

Enzymatic hydrolysis experiments were carried out with addition of commercially available enzyme, Accellerase 1500. After thawing, the treated solid pellet containing amorphous cellulose was neutralized to pH 4.8-5.0 by NH_4OH. The SSO samples were then brought to 50^oC before adding 30 FPU/ g glucan of Accelerase 1500. Both the pH value and temperature described were the optimum conditions for the Accelerase 1500 enzyme to mediate hydrolysis and release fermentable sugars as much as possible. The hydrolysis experiment was conducted in the benchtop shaking incubator. The incubator was set at 250 rpm to keep solids in constant suspension with the temperature of 50^oC for 72 hours. Samples were taken for sugar content at specified times: 0, 12, 24, 48 and 72 hours to measure sugar content. The relevant composition of the SSO was 33% (w/v) glucose, 19% (w/v) xylose and 3% (w/v) acetic acid.

Following enzymatic hydrolysis, batch soluble sugar fermentation was carried out to evaluate ethanol yields by performance of two different recombinant strains: *Z. mobilis* 8b and *S. cerevisiae* DA2416. Soluble sugars batch fermentation was performed in 250 mL serum bottles with 100 mL working volume and purged before being autoclaved. Temperature was maintained at 30^oC and pH was controlled at 6.0 by 1M potassium hydroxide (KOH) as suggested by previous study (Zhang et al., 2009).

Compositional analysis of the samples in duplicates for ethanol concentrations was carried out at 0, 12, 24 and 48 hours by HPLC. The metabolic ethanol yield, Ym was calculated as a mass of ethanol produced per mass of sugar consumed. The process ethanol yield, Yp was obtained by dividing the ethanol concentration by total sugar concentration in the feed medium.

The volumetric ethanol productivity was derived by ratio of ethanol concentration and time taken to complete fermentation (48 hours).

4.3. Results and Discussion

Due to its potential for industrial application, the SSO waste was chosen as the substrate to evaluate the values on sugar and ethanol yields by fermentation using *Z. mobilis* 8b and *S. cerevisiae* DA2416 strains. Detailed quantitative assessment on the composition of SSO waste was completed prior to this study (Mirzajani, 2009), and the results are presented in Table 4.1.

As seen in Table 4.1, approximately, more than half of the original sample is composed of moisture. Essential polymeric sugars in an oven dried SSO samples included: 33% glucose, 19% xylose, and about 9% of other sugars and 23% of lignin. These homogeneous samples with pH at 5.2-5.5 had approximately 80% of the food waste and a 20% of wood chips.

Enzymatic hydrolysis and fermentation experiments were next in the line to be conducted in sequence in the chosen SHF approach. The whole process usually takes five days to complete. The SSO samples pretreated by concentrated phosphoric acid (85% w/w) and ethanol (95% v/v) were hydrolyzed fast and glucan digestibility was found to be 72% after 24 hours and 90% after 72 hours. The high glucan digestibility seen in Figure 4.1 was achieved for the COSLIF-pretreated SSO with addition of 30FPU/ g glucan of Accelerase 1500.

This result was mainly attributed to drastic changes in surface morphology of intact and COSLIF-pretreated SSO samples. The intact SSO has obviously maintained its tight micro-fibril structure, while a COSLIF-pretreated sample evidenced homogeneous biomass as seen in our previous work (Bekmuradov et al., 2014a). The enzymatic glucose digestibility for pretreated

Table 4.1: Compositional analysis of SSO sample

Parameters	Average Value
A. Physical Properties	
Biomass as received	
pH	5.2 @ 25°C
Total Solids (TS)	44.33%
Moisture content	55.66%
Volatile organic compound (VOC) per dry mass	13.66%
Ash per dry mass	5.14%
Oven-dried and homogenized biomass	
pH	5.5 @ 25°C
Moisture content	6.60%
TS	93.40%
VOC	86.33%(TS)
Ash	13.60% (TS)
B. Sugars and Lignin	
(per oven-dried and homogenized biomass)	
Glucose	31%
Xylose	19%
Other sugars	9%
Total sugars	59%
Total Lignin	23%
C. Others	
Total Kjehldahl Nitrogen (TKN)	9198 µg/g
Extractives	7%
Calorific value	16961.6 kj/kg

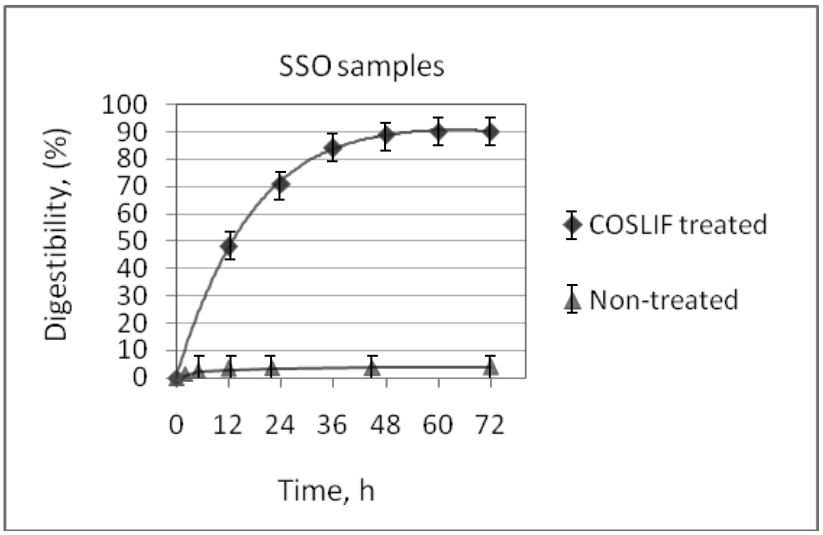


Figure 4.1: Glucan digestibility profiles for COSLIF treated and untreated SSO samples

COSLIF samples was calculated as described by Zhang et al. (2007). We hypothesized that almost all lignin have been removed from SSO waste sample during COSLIF and enzymatic hydrolysis phases. But it would be impractical to completely wash cellulose solvents out, as it requires a large amount of water. Negative effects of residual lignin on enzymatic hydrolysis may contribute to 1) enzyme adsorption by lignin, 2) obstruction of lignin on the surface of cellulose to that point when enzyme are not able to access cellulose (Collins, 2007; Zhu et al., 2009).

In a separate series of experimental evaluation, enzymatic hydrolysate obtained from COSLIF pretreament by batch culture fermentation with *Z. mobilis* 8b strain, was compared with *S. cerevisiae* DA2416. Figure 4.2 shows the glucose and xylose consumption trajectories for fermentation of the SSO pretreated samples.

As seen from Figure 4.3 both strains exhibited almost the same value of ethanol yields based on sugar consumed (0.48 g/g and 0.50 g/g) and the process yield on the total initial sugar concentration was 0.48 g/g for *Z. mobilis* 8b and 0.49 g/g for *S. cerevisiae* DA2416 (Table 4.2). After 72 hours, glucose is completely decomposed, while a small amount of xylose remains. Results show the main substrate for *Z. mobilis* 8b is glucose, while *S. cerevisiae* DA2416 decompose both glucose and xylose. Therefore the production of ethanol is higher for the

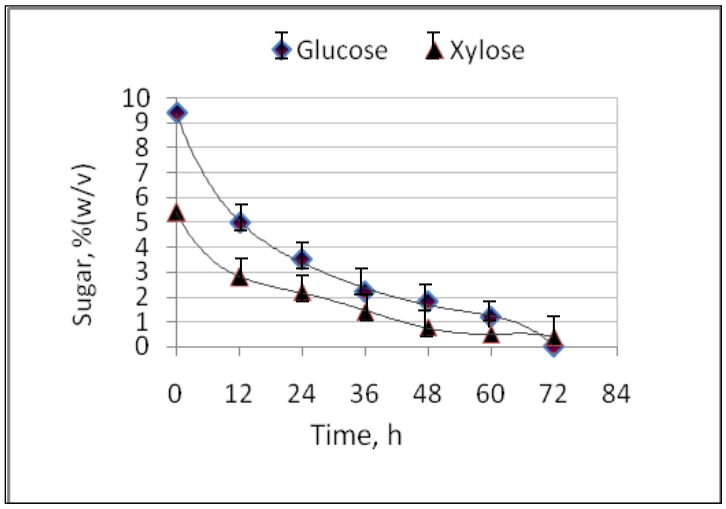


Figure 4.2: Sugar consumption profiles of the SSO pretreated hydrolysates during fermentation phase

S. cerevisiae DA2416 strain after glucose is used up. The significantly better performance of *S. cerevisiae* DA2416 compared to *Z. mobilis* 8b suggests a possible role of inhibitors other than acetic acid on bacterial growth in fermentation phase, for example phenolic compounds from lignin and etc. It is both well-known and documented (South et al., 1995; Rogers et al., 1997; Lawford and Rousseau, 2000) that ethanol is an inhibitor to xylose utilization by *Z. mobilis* 8b with ethanol concentration of 5.5%-6% (w/v) causing complete deceleration of the process.

In further fermentation assays with the *Z. mobilis* 8b strain, after 48 hours, 100% of glucose and 40% of xylose were consumed. On the other hand, in the enzymatic hydrolysate with *S. cerevisiae* DA2416, fermentation advanced more rapidly, with 100% glucose and 60% xylose consumed after the same period of time. The growth and fermentation parameters of this work are summarized in Table 4.2.

Table 4.2: Growth and fermentation parameters

Strains	*Z. mobilis* 8b	*S. cerevisiae* DA2416
Total amount of sugar, % (w/v)	14.8	14.8
Glucose, % (w/v)	9.5	9.5
Xylose, % (w/v)	5.3	5.3
Acetic acid, % (w/v)	1.0	1.0
Process yield, g/g	0.48	0.49
Metabolic yield, g/g	0.48	0.50
[1]Productivity, g/L·h	0.88	0.92
Ethanol yield, g/L	140	152

[1]Productivity data was based on fermentation time of 48 hours

Process yield was based on available sugars

Metabolic yield was based on sugar utilized

The fermentation was complete at 48 hours (Figure 4.3) with a final ethanol concentration of 4.5% (w/v) representing a volumetric productivity of 0.92 g/(L•h) and ethanol yield of 0.50 g/g or 96% theoretical maximum conversion efficiency for performance with *S.*

cerevisiae DA2416. The final ethanol concentration 3.5% (w/v) represented a volumetric productivity of 0.88 g/(L•h) and an ethanol yield of 0.48 g/g or 94% theoretical maximum conversion efficiency for performance with *Z. mobilis* 8b.

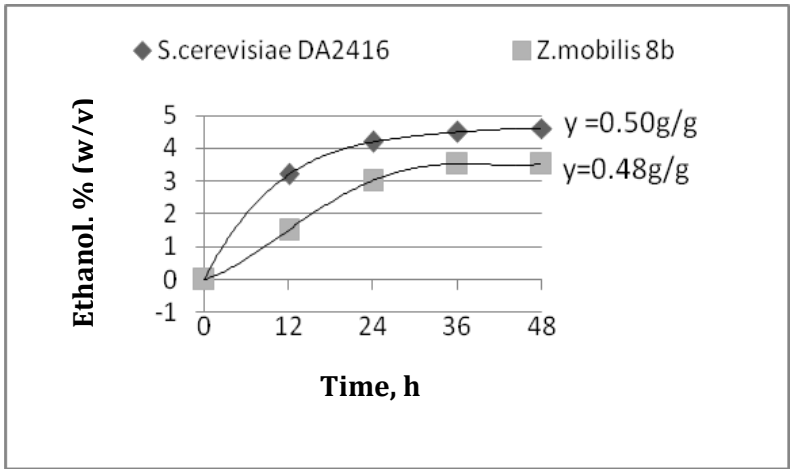


Figure 4.3: Comparative fermentation performance of both strains for ethanol production in time range of 48 hours

In summary, low bacterial activity in fermentation of SSO hydrolysate by *Z. mobilis* 8b may be attributed to many other factors, including: longer lag phase - an adaptation time for growth condition of chosen strain, low growth rate on SSO hydrolysate and lack of micronutrients such as nitrogen, phosphorus.

4.4. Conclusions

The SSO waste samples utilized in this research were pre-processed by the thermal screw press (TSP) and further used as substrates for all enzymatic hydrolysis and fermentation processes.

COSLIF pretreatments were applied for cellulose extraction from processed source separated organic waste. Results indicated the percent glucan conversion was considerable for COSLIF pretreated samples compared to untreated samples. This study demonstrated and affirmed that *S. cerevisiae* DA2416 outperformed *Z. mobilis* 8b on ethanol yields during fermentation process. However, a more comprehensive investigation on lignocellulosic usage with different enzymes and recombinant fermenting strains would be advantageous in biofuel field.

For supporting materials on this chapter please refer to Appendices A “Glucose and ethanol yields calculations” and D “Experimental procedures”.

CHAPTER 5. KINETIC MODEL FOR ETHANOL PRODUCTION BY RECOMBINANT S. CEREVISIAE STRAIN ON SOURCE-SEPARATED ORGANIC WASTE

Abstract

An existing kinetic model was adapted and modified to accommodate batch simultaneous saccharification and co-fermentation (SSCF) process on source-separated organic (SSO) waste by the recombinant strain, *Saccharomyces cerevisiae* DA2416. The model encompasses enzymatic hydrolysis and fermentation processes with competitive uptake of glucose and xylose in both stages. Enzymatic hydrolysis was featured with the addition of a commercially available enzyme, Accellerase 1500, to mediate the process. Pre-processing technologies, including the thermal screw press (TSP) and cellulose-organic-solvent-based lignocellulosic fractionation (COSLIF) pretreatments, were applied on the SSO waste to liberate all fermentable sugars. On average, the sugar yields, mainly in the form of glucose and xylose, from pretreated SSO waste by enzymatic hydrolysis was 90%. The kinetic model was tailored with experimentally-defined SSO parameters to evaluate the sugar and ethanol yields from SSO waste, and was found to be able predict ethanol production rate accurately with diminutive variance from experiments. Experimental results demonstrated that *S. cerevisiae* DA2416 produced more than 150 g/L ethanol, with ethanol yield of 0.50 g of ethanol/g potential sugar fed, in less than 5 days with 96% cellulose conversion. It was confirmed in this work that cellulose adsorption capacities along with hydrolysis rate constant have a high impact on sugar and ethanol formation.

This study provides important insights for investigation on the use of SSO waste for ethanol production by *S. cerevisiae* DA2416 and kinetic model is proven to be a useful tool to help facilitate future process optimization for pilot scale facilities.

5.1. Introduction

For many years, the main source of fuel for human society has come from fossil resources, which are not infinitive. Lignocellulosic biomass, on the other hand, is a promising alternative to fossil fuels and is considered the only foreseeable sustainable source of organic fuels and materials available to humanity (Shao, 2007). For example, lignocellulosic biomass such as agricultural residues along with the organic fraction of municipal solid waste is particularly attractive because of the low cost and considerable availability, estimated in Ragauskas et al. (2006) and Zhang et al. (2006) at approximately 200 billion metric tons worldwide. As a result, there is an increasing trend for biomass-derived fuel to provide a renewable alternative to conventional fuel for transportation sector. However, the current cost of conversion has been a bottleneck for commercial applications (Houghton et al., 2006). Among the strategies to reduce the processing costs are pretreatment and usage of all fermentable sugars present in biomass with technologies available on the market. Four approaches for cellulosic biomass processing featuring enzymatic hydrolysis have been reported: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) and consolidating bioprocessing (CBP) (Shao, 2007). There are four biologically mediated events identified in each approach, and they are cellulase production, cellulose hydrolysis, pentose fermentation and hexose fermentation (Wyman, 1999; Xiao et al., 2004).

SHF and SSF approaches are featured in many experimental designs for immediate and/or near-term implementation, while SSCF and CBP reqiure more research from deep-rooted problems in the process development (Shao, 2007). Chandrakant and Bisaria (1998) reported a major disadvantage of SHF was inhibition of cellulose hydrolysis by glucose, and as a result it

has not been possible to obtain glucose concentrations higher than 5.5% with this method. The SSF approach, featuring enzymatic hydrolysis and fermetation of hexose in one integrated step, considerably increases inhibition to cellulase by cellulose hydrolysis products (Wyman, 1999; Xiao et al., 2004) and is therefore limited in terms of process performance. The SSCF process is similar to SSF except that hexose and pentose fermentations occur in one step. Unlike SHF and SSF, the SSCF process offers potential for more streamlined processing and a lower capital cost (Chandrakant and Bisaria, 1998; Wyman, 1999) . Consequently, the SSCF approach was chosen in this work. SSCF has become more attractive with the emergence of new microorganisms that produce ethanol at a high yield from both glucose and xylose and reduce inhibition hydrolysis by xylose (Kim and Lee, 2005).

The CBP approach has a similar prospective but requires a higher temperature in the range of 50^{o}C and 60^{o}C for the enzmatic hydrolysis reaction than SSCF. Moreover, studies on CBP with native *Clostridium thermocellum* was found to produce ethanol with significant amounts of acetic acid, with very limited ability to utilize the xylose from the feed (Demain et al., 2005).

Normally the process design for ethanol production from lignocellulosic biomass is initiated with physiochemical pretreatment to increase the exposure of substrate to enzymatic hydrolysis, followed by the biological conversion of resulting sugars to ethanol by a chosen fermenting strain. A recombinant strain *S. cerevisiae* DA2416,which is capable of fermenting both glucose and xylose to produce ethanol at high yield, was used in this work. It has been observed that *S. cerevisiae* DA2416 has by-passed common problems with glucose repression, by taking advantage of an efficient xylose utilization pathway. As a result, the available sugars

are utilized more effectively, key fermentation parameters, such as overall ethanol yield and inhibition are improved further for better results in the fermentation phase.

A lignocellulosic biomass, made up of pre-processed SSO, which is a blend of approximately 80% organic “green bin” and approximately 20% of woodchips from construction/demolition waste, was used in this work as a feedstock for all enzymatic hydrolysis and fermentation processes. Results from previous studies on SSO, including a 6-month compositional analyses (Mirzajani, 2009) and chemical pretreatment (Ehsanipour, 2010), have shown that SSO can become a highly desirable potential substrate with unlimited availability and often at negative cost for future industrial applications.

An existing kinetic model was adapted from (Zhang, 2008) and modified in this work to predict batch SSCF on SSO waste by glucose and xylose utilizing strain *S. cerevisiae* DA2416. This model accounts for cellulose and hemicellulose enzymatic hydrolysis and competitive uptake of glucose and xylose. There are only a few published studies on the conversion of cellulose and hemicellulose via SSCF (McMillan et al., 1999; Teixeira et al., 2000; Kim and Lee, 2005) and only one kinetic model has been proposed in the literature (Zhang, 2008). The kinetic model development was based on a semi-mechanic rate equation for cellulose hydrolysis as initially proposed by South et al. (1995) and further modified by Shao (2007) and Zhang (2008) to accommodate cellulose and hemicellulose hydrolysis. The parameters presented in the literature (Shao, 2007; Zhang, 2008) were based on data for paper sludge only. Therefore, new values of interest such as the adsorption capacity, enzymatic hydrolysis constant, ethanol inhibition and ethanol yield were re-established with experimental data to accommodate the batch mode SSCF on SSO waste.

The main objectives of this paper are: a) to study the influence of different process variables presented in SSCF process configuration on pretreated SSO waste with chosen strain, *S. cerevisiae* DA2416; b) to develop an experimental kinetic model capable of predicting behavior of batch SSCF on SSO waste with different substrate concentrations: 20 g/L, 50 g/L and 100 g/L; and c) to compare performance of *S. cerevisiae* DA2416 on ethanol yield with other yeasts from the same family: *S. cerevisiae* RWB222 and *S. cerevisiae* D5A.

The overall objective of this work is to advance in the understanding and gaining knowledge for bioconversion process of converting SSO waste by SSCF approach for ethanol production.

5.2. Materials and Methods

The *S. cerevisiae* DA2416 recombinant strain used in this study was kindly provided by Dr. Yong-Su Jin from the Department of Food Science and Human Nutrition, University of Illinois, USA. It was kept at -80^{o}C in 30% (v/v) glycerol for storage. The enzyme complex Accellerase 1500 used in the hydrolysis experiments was donated by of Sigma Aldrich Corp., USA.

The SSO waste utilized in this work was initially pre-processed mechanically, under high temperature (120^{o}C) and pressure (50 bars) by a thermal screw press for 4-5 minutes to form a semi-dry stable biomass (Optimum waste and recycling systems Ltd, 2010). SSO samples were prepared as a heterogeneous substrate by blending 20%±2% construction/demolition wood waste in the form of the wood chips and addition 78%-80% of organic “green-bin” waste as in (Bekmuradov et al., 2014b). Optimum Waste Recycling Systems, Toronto, Canada, supplied the

biomass feedstock used in this work (Optimum waste and recycling systems Ltd, 2010). Prior to testing, the SSO waste was oven-dried at 45^{o}C-50^{o}C for 48 hours.

Chemical pretreatment with cellulose-organic-solvent-based lignocellulosic fractionation (COSLIF) was applied to release the glucose and xylose from the SSO (Bekmuradov et al., 2014a). Five grams of dry lignocellulose was placed in a 250-mL centrifuge bottle and then mixed with 40 mL of 85% concentrated phosphoric acid using a glass rod. The solid/liquid slurry was placed in a benchtop shaking incubator at 150 rpm and 50^{o}C±0.2^{o}C for two hours. One hundred mL of ethanol was then added to the contents and mixed well. After centrifugation at 7000 rpm at room temperature for 15 minutes, the supernatant was decanted. The solid pellet was then re-suspended with 200 mL of ethanol and centrifuged again at 7000 rpm. The supernatant again was decanted. Next, the solid pellet was re-suspended with 200 mL of distilled water and centrifuged two times and stored in a freezer (Rollin et al., 2009).

Enzymatic hydrolysis experiments were carried out with the addition of a commercially available enzyme, Accellerase 1500. After thawing, the treated solid pellet containing amorphous cellulose was neutralized to pH 4.8-5.0 by NH4OH. The SSO samples were then brought to 50^{o}C before addition of 30FPU/ g glucan of Accellerase 1500. Both the pH value and temperature described were the optimum conditions for the Accellerase 1500 enzyme to mediate hydrolysis to allow release of as much fermentable sugars as possible (Dowe and McMillan, 2008). The hydrolysis experiment was conducted in the shaking incubator (model MAXQ4450). The incubator was set at 250 rpm to keep solids in constant suspension with the temperature of 50°C for 72 hours. Samples were taken and measured for sugar content at specified times: 0, 12, 24, 48 and 72 hours. The relevant composition of the SSO was studied and reported in (Bekmuradov et al., 2014a).

The protein content of the SSO substrate was measured by a modified method of Lowry (Thermo Fisher Scientific Inc., 2011). Adsorption of cellulase onto SSO substrate was done by mixing them in an incubator shaker at 100 rpm in the Innova-40 shaker, at a temperature of 25^{o}C, in 10-mL glass tubes, under controlled pH and concentration of cellulase. Centrifugation of the reaction tube followed an incubation period, after which unbound cellulase present in the supernatant were decanted off. The amount of cellulase adsorbed onto a solid substrate was determined as the difference between the total amount of cellulase initially applied [E_{init}] and the amount of free cellulase in the solution [$E_{non\ ads}$]. The amount of free cellulase in the solution was measured by rapid UV spectrophotometer technique (Liu et al., 2011; Wang et al., 2012). The technique determines free cellulase concentrations in the solid SSO substrate suspension from the second derivative of the absorption spectra at 750 nm wavelength through calibration. Each data point in the plots was based on an average of 5 replicates.

The carbohydrate content of SSO was determined by quantitative saccharification (QS) based on 2 hours of incubation in 72% by weight H_2SO_4 at 30^{o}C (Ruiz and Ehrman, 1996; Moxley and Zhang, 2007) . The cell mass was determined by counting colony forming units on agar plates as described by Zhang et al. (2009).

Following enzymatic hydrolysis, batch soluble sugar fermentation was carried out to evaluate ethanol yields from SSO samples as a result of conversion using recombinant strain *S. cerevisiae* DA2416. Soluble sugar batch fermentation was performed in 250-mL serum bottles with 100 mL working volume. Temperature was maintained at 30^{o}C and pH was controlled at 6.0 by 1M potassium hydroxide (KOH) as suggested by previous study (Mohagheghi et al., 2004). Compositional analysis of the samples for ethanol concentrations was carried out at 0, 12, 24 and 48 hours) by HPLC (see Appendix E “Samples of HPLC runs for glucose and ethanol”).

The kinetic model adapted in this study uses a semi-mechanistic rate equation for cellulose hydrolysis as proposed by South et al. (1995) and further modified in Zhang et al. (2009). The parameters presented in Zhang's kinetic model were based on cellulose and hemicellulose hydrolysis for pretreated paper sludge. In this study adsorption parameters were re-established based on overall carbohydrate content of pretreated SSO waste. The binding capacity or specific capacity of the carbohydrate component for cellulase of SSO samples was obtained using Langmuir isotherms. The remaining cellulose hydrolysis parameters were as reported by Zhang (2008) . Experimental data on glucose and xylose consumptions and growth parameters were fitted using the non-linear function of Polymath 5.1 (Polymath Software: Willimantic, CT, USA). All other parameters were dynamically fitted with the curve fitting function in the Berkeley Madonna computer program with a fourth-order Runge-Kutta algorithm. Runs were performed on a standard laptop.

A sensitivity analysis was carried out based on the least-square method (Stigler, 1986; Bretscher, 1995). It was performed to test the impact of the value of important parameters on the model prediction of ethanol production if the values were changed to ±10% from those obtained from experiments. The analysis was performed to determine the difference between experimental data and modified kinetic model predictive ability.

5.3. Results and Discussion

Due to its prospect for commercial application, SSO waste was chosen as the substrate to evaluate the values on sugar and ethanol yields by fermentation using *S. cerevisiae* DA2416 strain.

Approximately, more than half of the original sample was composed of moisture. Essential polymeric sugars in the oven dried SSO samples included: 31% glucose, 19% xylose, and about 9% of other sugars and 23% of lignin. These homogeneous samples had a pH range of 5.2-5.5. The SSO samples pretreated by concentrated phosphoric acid (85% w/w) and ethanol (95% v/v) were hydrolyzed and glucan digestibility was found to be 72% after 24 hours and 90% after 72 hours. The high glucan digestibility was achieved for the COSLIF-pretreated SSO with addition of 30 FPU/ g glucan of Accellerase 1500 (Bekmuradov et al., 2014b).

Recognizing that cellulase mixtures contain a mixture of cellulase and hemicellulose portions which bind to cellulose and hemicellulose, adsorption capacity constant "σ" was recalculated using the modified method of Lowry and resultantly they were in the range of 0.264 to 0.280. Similarly, the binding capacity C_S was determined as between 0.442 g protein/g carbohydrate and 0.466 g protein/g carbohydrate using Langmuir isotherms. Cellulose hydrolysis rate constant – k (1/h) had range values of 0.662 and 0.725. The remaining cellulose enzymatic hydrolysis parameters were adapted from Zhang (2008). Adsorption of Accellerase 1500 cellulase to the SSO waste samples was evaluated after hydrolysis was allowed to proceed for specified time, (6, 12, 24, 36, 48, 60 and 72 hours) resulting in various values for fractional conversion up to 85% as shown in Figure 5.1.

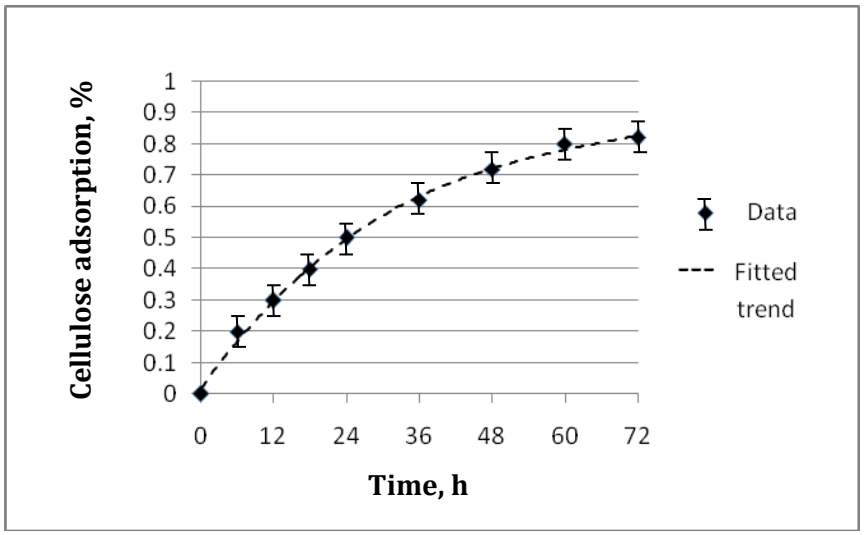


Figure 5.1: Cellulose adsorption data for pretreated SSO samples

The predictions were confirmed by experimental data obtained during the addition of Accellerase 1500 enzyme complex to SSO samples throughout the course of hydrolysis. The predictions over the time of reaction are almost the same. The good fit obtained in this study suggests that as for the SSO samples it seems reasonable to assume a constant adsorption capacity normalized to the amount of cellulose remaining and there is no reason to hypothesize adsorption affinity as a function of a conversion.

Adsorption parameters K_S and σ_S in Table 5.1 were then fit to the data of all conversions by minimizing the sum of squares for the predicted and experimental data. The new adsorption parameters with conversion data were used to fit the parameters *k*, *c* and *e* in the cellulose rate equation by South et al. (1995). The values of parameters are presented in Table 5.1.

Table 5.1: Average parameter values for SSCF of the SSO.

K_S	0.466	This work
σ_S	0.280	This work
k	0.725	This work
e	0.516	This work
c	0	Shao, 2007

With parameter values in hand for adsorption, hydrolysis and fermentation, a pre-existing kinetic model for SSCF was modified to account newly defined SSO feedstock constants. In view of this, we selected a simple correlation model reported by Zhang (2008) as in the following equation:

$$r_{Xn} = \frac{XI}{GI} \times r_{Gn} \qquad (1)$$

where r_{Gn} and r_{Xn} - formation of glucan and xylan respectively, *GI, XI* - initial glucan and xylan concentrations.

Equation (1) above describes the correlation of glucan and xylan hydrolysis and is derived from the simple relationship of $X_1=X_2$ in which: X_1 and X_2 are the conversion of glucan and xylan respectively. To understand the enzyme hydrolysis performance with the *S. cerevisiae* DA2416 strain, the percentage of glucan and xylan conversion was calculated. Glucan and xylan used in SSO in this work were converted to monomeric sugars at almost the same rate by Accellerase 1500. The average glucan conversion to monomer sugars was 96%, and the average xylan conversion to monomer sugars was 94% at 30^{o}C. However, we observed a slightly higher

residual xylose accumulation than residual accumulation of glucose during experimental tests. The slower consumption rate of xylose than glucose was consistent with the fermentation of soluble sugars in other studies as well utilizing *S. cerevisiae* (Kuyper et al., 2005; Zhang and Lynd, 2010).

To begin SSCF runs, Accellerase 1500 enzyme at 30FPU cellulase were mixed with yeast innocula and added into the vessel to increase the sugar's accessibility to cells during the mass transfer limited period. High glucan digestibility (approximately 90%) was achieved. Batch soluble sugar fermentation experiments were carried out to find the fermentation related constants, exclusive ethanol inhibition and yield in SSCF kinetic model by performance of recombinant strain *S. cerevisiae* DA2416. In *S. cerevisiae* strains, there are a large number of genes encoding hexose transporters (Reifenberger, 1997), which are also believed to function with low affinity xylose transporters in recombinant xylose utilizing *S. cerevisiae strains* (Sedlak and Ho, 2004). Based on this examination, a competitive substrate inhibition model for growth in glucose and xylose by *S. cerevisiae* DA2416 was chosen to capture the growth kinetics. Inhibition of growth and fermentation has been described using different equations in the literature, including exponential inhibition, linear inhibition, and linear inhibition beyond threshold (van Uden, 1989). Among them, a threshold linear inhibition model was chosen because it fit best with the data. A threshold linear inhibition model equation (2) as described elsewhere (South et al., 1995) accounts for glucose fermentation with an additional term representing sugar uptake from xylose and inhibition from ethanol:

$$\mu_{Gl} = \left[\frac{X \cdot \mu_{Gl}^{Max} \cdot G_l}{K_{Gl} + G_l + I_1 \cdot X_l}\right] \times \left(1 - \frac{Eth}{Eth_{Gl}^{Max}}\right)^{f1} \qquad (2)$$

where μ_{Gl}^{Max} and Eth_{Gl}^{Max} - maximum specific growth rate and maximum ethanol concentration for growth on glucose respectively; G_l, X_l, Eth - concentration of glucose, xylose and ethanol; K_{Gl}, I_1, f^1 - related constants.

The rate of formation of xylose was described by South et al. (1995), similar to that used for glucose formation shown in the following equation:

$$\mu_{Xl} = \left[\frac{X \cdot \mu_{Xl}^{Max} \cdot (X_l - X_{lT}}{K_{Xl} \cdot X + X_l + I_2 \cdot G_l}\right] \times \left(1 - \frac{Eth}{Eth_{Xl}^{Max}}\right) \qquad (3)$$

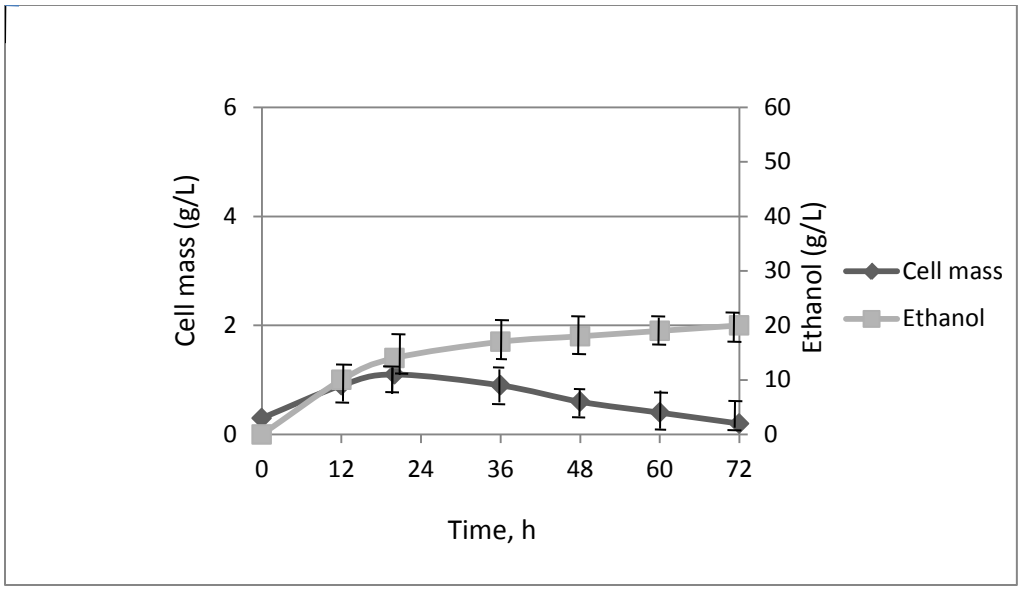
where $\mu_{Xl}^{Max}, Eth_{Xl}^{Max}$ - maximum specific growth rate and maximum ethanol concentration for growth on xylose respectively; K_{Xl}, I_2 - related constants; X_{lT} - threshold concentration.

The well-known phenomenon of declining hydrolysis rate (Zhang, 2009) as the reaction progresses was modeled using the following empirical equation:

$$k(x) = k[1 - x(t)]^e + c \qquad (4)$$

Values for inhibition factors I_1 and I_2 in this work were found to be 0.108 and 6.032 respectively, indicating that the inhibition of xylose utilization by glucose is more than 50 times stronger than the inhibition of glucose utilization by xylose.

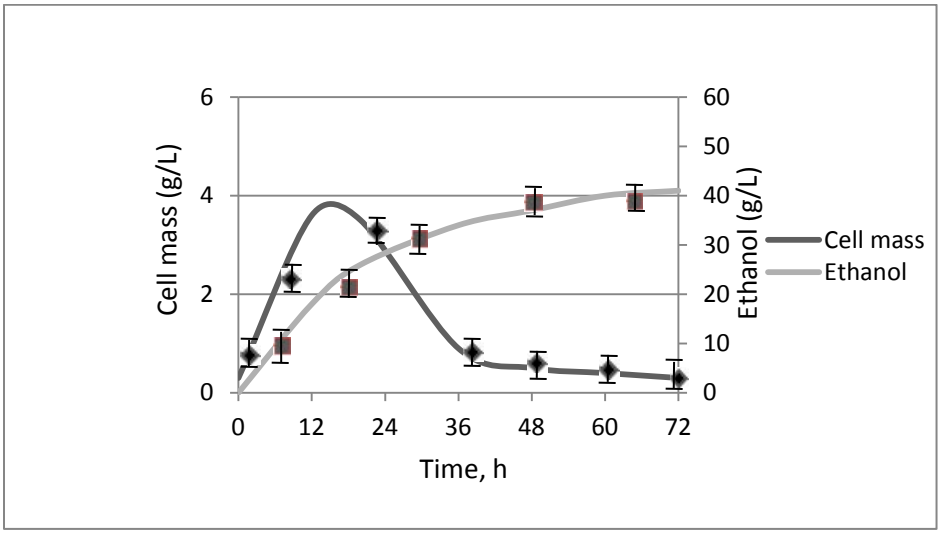
In order to test the SSCF performance on SSO feedstock with newly redefined constants, batch fermentations were carried out in a separate series of experimental evaluation at different substrate concentrations of 20 g/L, 50 g/L and 100 g/L with the enzyme loading of 30FPU cellulase. Initial substrate concentration for model calibration is kept low, below 10 g/L, so, that



Cell mass-expt Cell mass-model

Ethanol-expt Ethanol-model

Figure 5.2: Experimental data and kinetic model prediction for SSO samples (substrate concentration 20 g/L).



Cell mass-expt Cell mass-model

Ethanol-expt Ethanol-model

Figure 5.3: Experimental data and kinetic model prediction for SSO samples (substrate concentration 50 g/L)

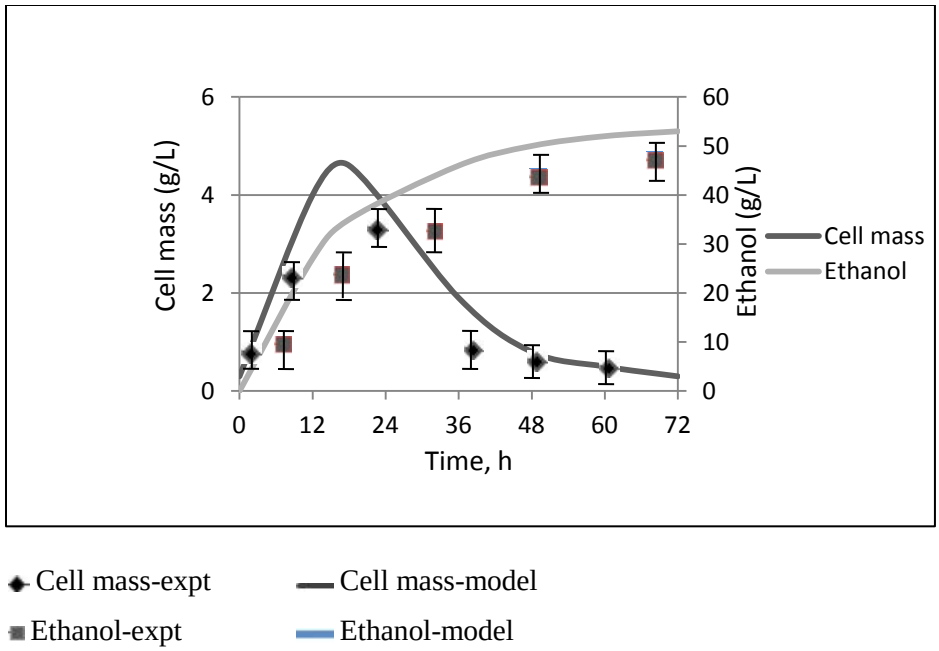


Figure 5.4: Experimental data and kinetic model prediction for SSO samples (substrate concentration 100 g/L)

effect of produced ethanol can be ignored. The model accurately predicts the sugar and ethanol concentration along with cell mass for 20 g/L and 50 g/L (experimental data shown with error margins) as shown in Figures 5.2, 5-3, but not for 100 g/L (Figure 5.4).

In higher substrate concentration mode, reaction deviated from experimental values as shown in Figure 5. 4, suggesting ethanol inhibition is a factor causing this discrepancy. There are also other reasons attributed to it, for example, enzyme deactivation or enzyme adsorption by lignin and/or obstruction of lignin on the surface of cellulose to the point that enzymes are not able to access cellulose (Collins, 2007; Faye, 2010).

Another set of experiments was conducted to assess the contribution of ethanol inhibition to the loss of cell viability. We expected good cell viability at lower SSO sample concentrations if inhibition was a major factor for cells lost. It was noticed in experiments that ethanol

concentration was a major factor of declining cell viability, but at the same time, one or more factors other than ethanol inhibition that were not yet determined might contribute to the loss viability in SSCF using *S. cerevisiae* DA2416. Table 5.2 below presents the results of sensitivity analysis.

In the sensitivity analysis, the sum of variance between the measured ethanol and the ethanol predicted by the model were calculated. Single parameters were varied to ±10% of the experimental values and responses S^* were calculated as absolute values based on the least-square method (Stigler, 1986; Bretscher, 1995), where S^* is defined as in the following equation:

$$S^* = \left|\frac{V_2 - V_1}{V_1}\right| \qquad (5)$$

where V_2- sum of variance between measured and predicted values from kinetic modelwith a single model constant changed to ±10%; V_1 - sum of variance between the measured and predicted value with all kinetic model constants unchanged from their measured values.

As seen from Table 5.2, cellulase adsorption constant C_S had the highest response value followed by the cellulose enzymatic hydrolysis constant *k*, among the enzymatic hydrolysis constants. From the results, a 10% decrease of C_S is almost 3 times more sensitive than a 10% increase, demonstrating that increase in enzyme loading will be more effective than increase in substrate concentration for ethanol yield. Among the microbial growth related constants, the ethanol yield from glucose and xylose exhibited the highest sensitivity, with ethanol tolerant related constants showing moderate sensitivity.

Finally, a comparison of the performance of *S. cerevisiae* DA2416 with different *S. cerevisiae* strains in terms of the ethanol production and glucan and xylan conversions are performed and the results are presented in Table 5.3. The results in Table 5.3 shows the highest

Table 5.2: Sensitivity analysis of kinetic parameters for SSO used in this work

Parameter	Value	Unit	Source	S^* value (10%) increase	S^* value (10%) Decrease
Enzymatic hydrolysis constants					
C_S	1.29	g protein/ carbohydrate	This work	1.99	5.27
k	0.69	1/h	This work	1.26	0.32
K_S	0.466	L/g	This work	0.44	0.34
K_C	663.4	l/h	Shao, 2007	0.03	0.03
K_{SP}	50.35	dimensionless	Phillipidis, 1993	0.35	0.24
m	0.537	dimensionless	Shao, 2007	0.21	0.32
Microbial growth related constants					
$Y_{Eth/Gl}$	0.50	g ethanol/g glucose	This work	5.42	0.46
$Y_{Eth/Xl}$	0.46	g ethanol/g xylose	This work	0.53	0.29
f_1	2.97	dimensionless	This work	0.32	0.30
I_1	0.11	dimensionless	This work	0.02	0.02
I_2	6.03	dimensionless	This work	0.02	0.02
Eth_{Gl}^{Max}	87.8	g/L	This work	0.36	0.32
Eth_{Xl}^{Max}	62.3	g/L	This work	0.24	0.48

S^* - sensitivity factor, represents the response to a given 10% increase or decrease of single constant value

ethanol production achieved for *S. cerevisiae* DA2416 by SSCF on SSO, as compared to those by *S. cerevisiae* RWB222 and *S. cerevisiae* D5A as tested on paper sludge. Experimental results demonstrated *S. cerevisiae* DA2416 produced an ethanol yield of 0.50 g of ethanol/g potential sugar fed on SSO in less than 5 days with 96% cellulose conversion totalling in 150 g/L ethanol.

All strains exhibited almost the same value of glucan and xylan conversion. Performance of *S. cerevisiae* DA2416 on SSO has a higher ethanol production, most probably due to a higher enzymatic activity and higher tolerance to inhibitors than other two strains. Although, different substrates, including paper sludge should be tested to validate the DA2416 strain in the future work.

Table 5.3: Comparison performance of different *S.cerevisiae* strains

S. cerevisiae strain:	DA2416	RWB222	D5A
g ethanol/g sugar consumed	0.48±0.01	0.40±0.01	0.44±0.01
g ethanol/g sugars fed	0.50±0.01	0.35±0.01	0.33±0.01
final glucan conversion	0.96±0.01	0.89±0.02	0.94±0.01
final xylan conversion	0.94±0.02	0.88±0.02	0.93±0.02
% ethanol production compare to D5A	152	105	100

S. cerevisiae DA2416 @ 30^{O}C – this work

S. cerevisiae RWB222 @ 30^{O}C – (Zhang and Lynd, 2010)

S. cerevisiae D5A @ 37^{O}C– (Zhang and Lynd, 2010)

5.4. Conclusions

The SSO waste samples utilized in this research were pre-processed by the TSP and further used as substrates for all COSLIF, enzymatic hydrolysis, and fermentation processes.

COSLIF pretreatment was applied for cellulose and sugars extraction from pre-processed SSO waste. Fermentation results demonstrated S. *cerevisiae* DA2416 produced more than 150 g/L ethanol with ethanol yield of 0.50 g of ethanol/g potential sugar fed on SSO in less than 5 days. It was demonstrated that a kinetic model with integrated values of experimentally defined SSO feedstock constants was successful in predicting the ethanol yield accurately with diminutive variance from experiments. The cellulose adsorption constant, ethanol tolerance, and ethanol yield played very important roles in the fermentation process. It was identified in this work as confirmed fact that glucose and xylose utilization were inhibited by each other to a certain extent or region, specifically in the region with low sugar concentration level. Typically, ethanol production rate in SSCF with low sugar concentrations is highly depends on enzymatic hydrolysis rate. As a consequence, at substrate concentration levels (e.g. between 20 g/L up to 50 g/L), the rate predicted by kinetic model was in good agreement with experimental data and showed the sign of deviation at concentration rate of 100 g/L, suggesting that ethanol inhibition was a main cause of discrepancy. Therefore, the discrepancy between experiments and kinetic model predictions, particularly at high substrate concentrations, needs to be examined more comprehensively.

Additionally, a good fermenting strain should have the ability to withstand ethanol toxicity and common inhibitors such as aliphatic acids, furan aldehydes, furfural and inorganic compounds.

This study demonstrated and affirmed that *S. cerevisiae* DA2416 is a promising strain for SSO substrate in SSCF. In the future, the kinetic model used should be expanded to introduce the inference of lignin in lignocellulosic biomass.

For supporting materials on this chapter please refer to Appendices B “Absorption capacity calculations” and C “Berkeley Madonna computer program code and tabulated data”.

CHAPTER 6. CONCLUSIONS AND FUTURE WORK RECOMMENDATIONS

6.1. Conclusions

This thesis sought to gain a better understanding of bioconversion process of pretreated SSO waste by SHF and SSCF process configurations via experimental work and kinetic modeling. It was found in this study that: a) SSO is an excellent feedstock material for ethanol conversion with total fermentable sugars available in the samples for conversion in the range of 40%-41%, however, high lignin content in the samples, 27%-30%, emphasize on the need for pretreatment; b) modified COSLIF method is proven to be a viable method for pretreatment of SSO feedstock with the efficiency improved by 20% using ethanol instead of acetone. On average, glucose yield from SSO samples pretreated by modified COSLIF was approximately 90% versus 70% by standard method after enzymatic hydrolysis; c) *S. cerevisiae* DA2416 is a promising strain for SSO substrate in SSCF runs with ethanol yield of 0.50 g of ethanol/g sugar fed or 96% substrate conversion ; and d) kinetic model with newly interpolated values of experimentally defined SSO feedstock constants is able to predict the batch fermentation process well at substrate concentration range between 20 g/L and 50 g/L, but noticeably deviated at substrate concentration of 100 g/L.

The major parts of this study include: a) experimental work of biochemical pretreatment of SSO waste as a valuable source of cellulosic biomass for ethanol production only; b) comparative study of ethanol production from pretreated by modified COSLIF method on SSO samples by two different microorganisms, bacteria - *Z. mobilis* 8b and recombinant yeast - *S. cerevisiae* DA2416; and c) alteration of existing kinetic model with newly experimentally

defined SSO feedstock constants to predict batch SSCF on SSO waste with different substrate concentrations. The findings of this thesis are described below.

6.2. Experimental Work

The experimental work for this thesis consisted of three categories: experiments for SSO pretreatments, which include thermal screw press followed by COSLIF, enzymatic hydrolysis, fermentation, and kinetic modeling.

"Pre-processing" technologies, including the TSP and COSLIF were used with respect to pretreatments. An improvement on the standard method of COSLIF pretreatment was developed based on lower enzyme loading and using an ethanol washing instead of acetone. Results indicated the percent glucose conversion was considerable (90% versus 70%) for the modified COSLIF method with a significant glucose yield approximately 90%.

High glucan digestibility of the pretreated SSO was accredited to drastic changes in the supramolecular structure of the biomass before and after the COSLIF pretreatment, observed by the SEM in this study (Figure 3.5; Chapter 3).

The glucose digestibility of the pretreated standard COSLIF sample was approximately 70% as presented in (Figure 3.3; Chapter 3) with high enzyme loading of 60FPU and acetone washing. On the other hand, with a lower enzyme loading, 30FPU and ethanol washing, it reached 90% digestibility after 36 hours. This suggests that by removing hemicelluloses and lignin barriers, there was an increase in accessibility to the cellulose change by the cellulobiose, while also reducing the competitive inhibition of xylan to endo-glucanase. The effectiveness of the enzymatic hydrolysis was gauged by assessing the potential inhibitory factors and effects of

fermentation. The high ethanol yield presented in (Figure 4.3; Chapter 4) indicated very little inhibitors were present in the hydrolysates that were pretreated by the modified COSLIF method.

In cases of kinetic modeling, experiments were performed to obtain new data (cellulose adsorption constant, enzymatic hydrolysis rate, ethanol yields from glucose and xylose, and ethanol tolerance) for comparison with model predictions. The good agreement between data and kinetic model fit indicated that model was robust and accurately predicted the sugar and ethanol concentration along with cell mass for 20 g/L and 50 g/L (Figures 5.2 and 5.3; Chapter 5), but not for 100 g/L. In higher substrate concentration mode, reaction deviated from experimental values as shown in (Figure 5.4; Chapter 5) suggesting that there were other reasons attributed to it, e.g. enzyme adsorption by lignin and/or obstruction of lignin on the surface of cellulose to that point when enzyme were not able to access cellulose.

6.3. SSO Waste as a Lignocellulosic Feedstock for Ethanol Production

Big metropolitan cities around the world have been experiencing a challenge over the past few years regarding their waste problems, and having inadequate infrastructure for effective waste management practices. The city of Toronto in Canada is a perfect example with its waste problems that have recently become more critical. With the anticipation of landfills' closure and environmental concerns, the city of Toronto requires more efforts in developing economically feasible strategies for dealing with its waste. One of these strategies is using organic fraction of municipal waste such as SSO, which has a great potential for production of value added fuels and chemicals.

The experimental results showed a relatively high amount of carbohydrates in the SSO samples (glucose - 31% and xylose - 19%), indicating a great potential of SSO to be utilized as an ethanol production feedstock instead of using other cellulosic feedstocks such as herbaceous energy crops. A technology for the biological conversion of SSO to ethanol was proposed based on the current techniques and the results from the characterization study on SSO. It included physical pretreatment by TSP machine, chemical by COSLIF, enzymatic hydrolysis with addition of enzyme complex Accelerase 1500 and fermentation by two recombinant strains: *Z. mobilis* 8b and *S. cerevisiae* DA2416.

6.4. Comparative Ethanol Productivities of two Different Recombinant Fermenting Strains on the SSO Waste

The SSO waste samples utilized in this research were pre-processed by the thermal screw press and further used as substrates for all enzymatic hydrolysis and fermentation processes. COSLIF pretreatments were applied for cellulose extraction from processed source separated organic waste. Results indicated the percent glucan conversion was considerable for COSLIF pretreated samples compared to untreated samples (Figure 4.1; Chapter 4). This study demonstrated that *S. cerevisiae* DA2416 outperformed *Z. mobilis* 8b on ethanol yields during fermentation process. At 48 hours of fermentation, ethanol yield was equivalent to 0.48 g of ethanol produced per gram of SSO biomass by *Z. mobilis* 8b and 0.50 g of ethanol produced per gram of SSO biomass by *S. cerevisiae* DA2416. However, a more comprehensive investigation on lignocellulosic usage with different enzymes and recombinant fermenting strains would be advantageous in bio-fuel field.

6.5. Kinetic Modeling

A kinetic model adapted and altered in this work to predict batch SSCF on SSO waste by new recombinant strain *S. cerevisiae* DA2416. To the knowledge of the author, this is first kinetic model, which was specifically applied for SSO feedstock. The kinetic model included adapted correlation of cellulose and xylan enzymatic hydrolysis, competitive substrate uptake, xylose utilization kinetics, and accelerated cell death due to ethanol exhibition. The most important parameters for enzymatic hydrolysis (adsorption capacity and enzymatic hydrolysis constant) and for fermentation (ethanol yield and maximum ethanol tolerance) were measured or re-calculated to address new changes for SSO feedstock. The newly re-developed kinetic model accurately predicted the sugar and ethanol concentrations along with cell mass for 20 g/L and 50 g/L (Figures 5.2 and 5.3), but not for 100 g/L (Figure 5.4). There were a several causes for that: enzymes deactivation; high concentration of inhibitors presented in SSO; and high concentration of ethanol in the tested broth.

6.6. Future Work Recommendations.

Two lines of further study are recommended:

1) More research should be done with different enzyme complexes to reduce or avoid by-products production such as ethyl, methane, and acetic acid. As for Accelerase 1500 enzyme complex, it is unknown wither it attaches to hemicelluloses accurately. An enzyme complex with a higher ethanol resistance should be sought.
2) The kinetic model re-developed in this work can predicts the sugar and ethanol concentrations from SSO on SSCF by *S. cerevisiae* DA2416 for the low substrate

concentration mode. The discrepancy between kinetic model prediction and experimental data at higher substrate concentration needs to be examined further. Besides the limitation at high substrate concentration, future kinetic models should address the interference of lignin for its application as well as an introduction of other feed stocks pretreated by different approaches. Other real world substrates should be tested by different *S. cerevisiae* fermenting microorganisms.

APPENDIX A: GLUCOSE AND ETHANOL YIELDS CALCULATIONS

Glucose yield from SSO by Quantitative Saccharification (QS)

Date: Aug 7, 2013

Table 1: Moisture Content Measurement

Sample	Mass of Foil (g)	Mass of Foil + Sample (g)	Mass of Sample (g)	Mass of Dried Foil + Sample @ 105°C (g)	Mass of Dried Sample (g)	Total Solid (%)	Moisture Contents (%)
1	1.330	3.330	2.000	2.450	1.120	56.00	44.00
2	1.320	3.320	2.000	2.510	1.190	59.50	40.50
3	1.320	3.320	2.000	2.270	0.950	47.50	52.50
4	1.320	3.320	2.000	2.360	1.040	52.00	48.00
5	1.320	3.320	2.000	2.370	1.050	52.50	47.50
Avg						53.50	46.50

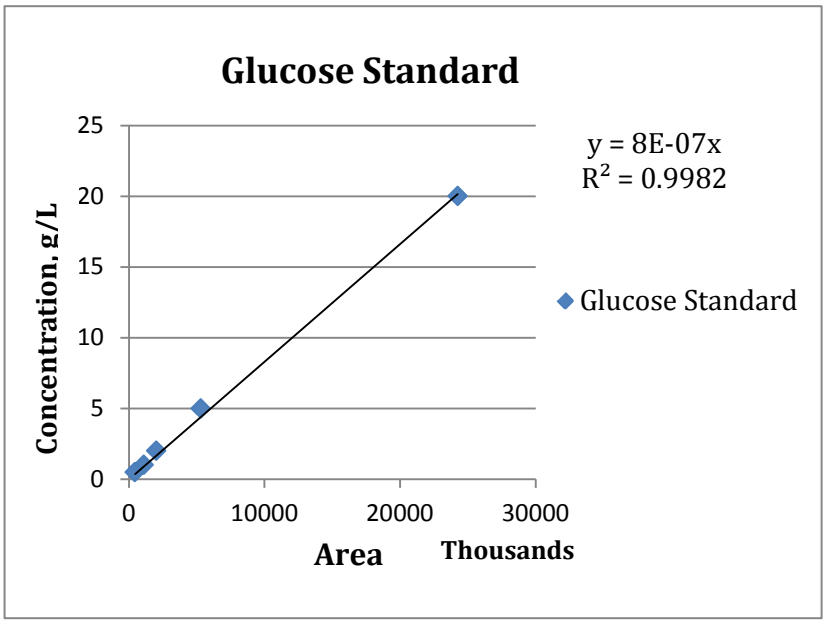


Figure 1: Glucose standards plot

Table 2: Glucose responses vs different concentrations

Standards	Conc. g/L	Glucose Area(µV.S)
S1	20	24250902.38
S2	5	5311241.79
S3	2	2020925.80
S4	1	1096904.40
S5	0.5	449967.00

Table 3: Glucose yield calculations

Bottle #	Sample Name	Glucose (HPLC)	Glucose (HPLC)	Glucose (HPLC)	Corrected Glucose	Glucose in 0.3 g QS Sample	Pretreated SSO/ Bottle (Avg)	Pretreated SSO/ Bottle w/o Moisture	Glucose per Pretreated Dry SSO Bottle	Glucan per Pretreated Dry SSO Bottle	Glucan Yield per 5 g SSO Bottle
		Area(µV.S)	Con. (g/L)	Con. (g/L)	(g/L)	(g)	(g)	(g)	(g)	(g)	(%)
1	QS1	609238.46	0.49	0.44	0.65	0.056	5.000	2.675	0.503	0.453	16.9
	QS2	482873.30	0.39								
2	QS3	391310.31	0.31	0.30	0.45	0.039	5.000	2.675	0.348	0.313	11.7
	QS4	364344.20	0.29								
3	QS5	418668.64	0.33	0.32	0.47	0.041	5.000	2.675	0.363	0.327	12.2
	QS6	369992.40	0.30								
4	QS7	520750.20	0.42	0.41	0.61	0.053	5.000	2.675	0.471	0.424	15.8
	QS8	502271.16	0.40								
									AVG =	0.379	14.2
										StdDev =	2.2

Sample Calculation:

- % SRS (Sugar Recovery Standards) = (conc. detected by HPLC, g/L) x 100/(known conc. of sugar before hydrolysis, g/L) = 6.74 g/L x 100/ (0.1/0.01) g/L = 67.4%; 87 ml = 0.087 L – 3 ml acid + 84 ml DDW
- Corrected Glucose = 0.44 g/L / 0.674 (SRS) = 0.653 g/L
- Glucose in 0.3 g QS Sample = 0.65 g/L x 0.087 L = 0.0565 g
- Pretreated SSO per bottle = 5 g
- Pretreated SSO per Bottle without Moisture = 5 g x 0.535 (%TS from Moisture Contents table) = 2.675 g
- Glucose per Pretreated Dry SSO Bottle = (2.675 g x 0.0565 g) / 0.3 g = 0.504 g
- Glucan per Pretreated Dry SSO Bottle = 0.504 g x 0.9 = 0.454 g
- Glucan Yield per 5 g SSO Bottle = 0.454 g x 100 / 2.675 g = 16.96 %

Cellulase Activitiy of Accelerase 1500

Date: April 15, 2013

Table 4: Glucose standards

Standards #	Glucose stock (ml)	Citrate Buffer (ml)	Dilution	Glucose (mg/0,5 ml)	Abs @540nm
S1	1	0.5	1/15	3.35	0.605
S2	1	1	1/2	2.5	0.473
S3	1	2	1/3	1.65	0.317
S4	1	4	1/4	1	0.179

Table 5: Glucose responses vs different concentrations

Dilution #	Citrate buffer (ml)	1:20 Enzyme (ml)	Conc.
1	33	7	0.00875
2	34	6	0.0075
3	36	4	0.005
4	37	3	0.00375
5	38	2	0.0025

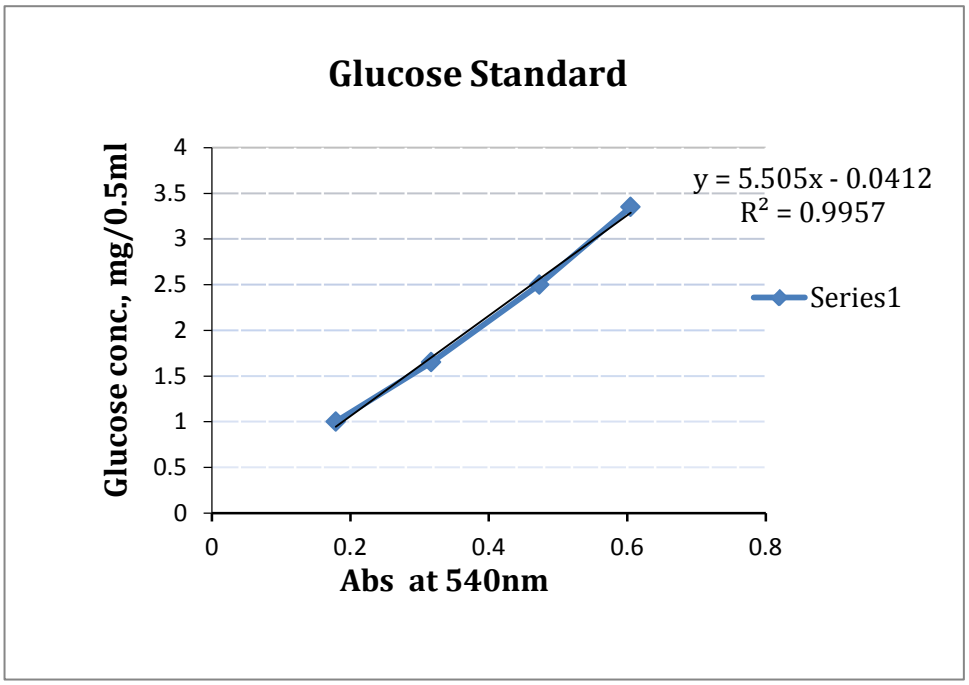


Figure 2: Glucose standards plot

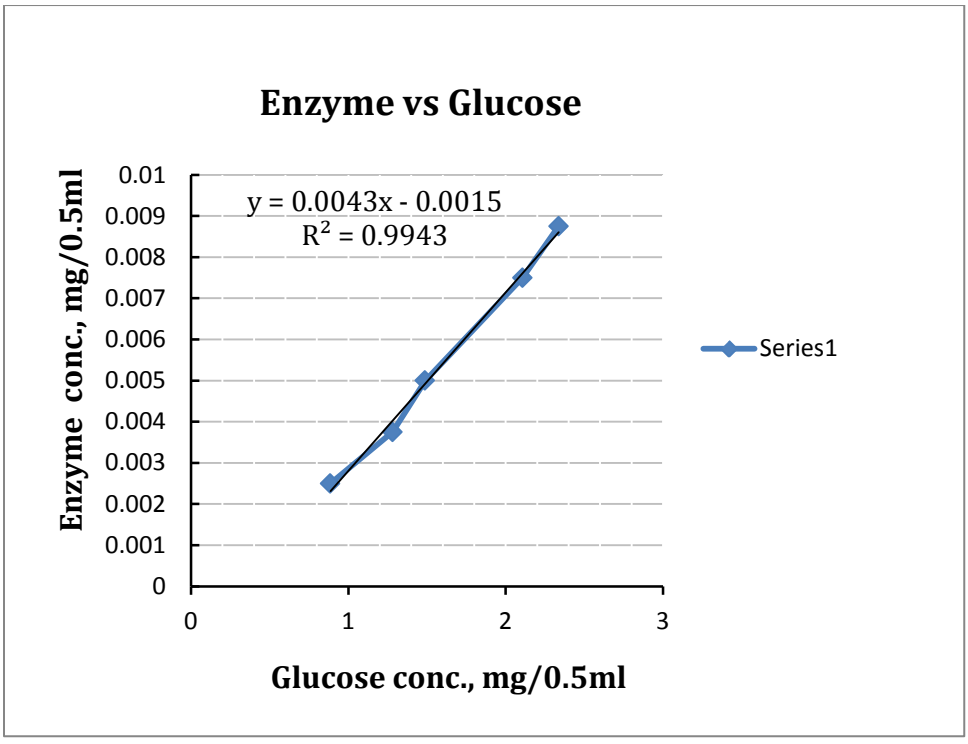


Figure 3: Enzyme vs Glucose plot

Table 6: Glucose readings vs dilutions factor

Dilution #	Sample #	Abs @ 540nm	Average Abs	Enzyme blank	Substrate blank	Corrected Abs @540nm	Glucose (mg/0.5ml)
	1.1	0.419					
1	1.2	0.42	0.433	0.001	0	0.432	2.337
	1.3	0.46					
	2.1	0.374					
2	2.2	0.396	0.391	0.001		0.390	2.108
	2.3	0.404					
	3.1	0.266					
3	3.2	0.277	0.278	0		0.278	1.488
	3.3	0.29					
	4.1	0.259					
4	4.2	0.219	0.240	0		0.240	1.282
	4.3	0.243					
	5.1	0.176					
5	5.2	0.154	0.168	0		0.168	0.886
	5.3	0.175					

Table 7: Enzyme concentration vs glucose

Dilution #	Enzyme conc	Glucose (mg/0.5 ml)
1	0.00875	2.337
2	0.0075	2.108
3	0.005	1.488
4	0.00375	1.282
5	0.0025	0.886

Calculation of FPU from graph at 2.0 glucose using equation y = (0.004x2 - 0.001)

0.0075 - X	2.108 - 2.0
0.0075 - 0.005	2.108 - 1.488

X = 0.00706 → FPU = 52.4

Enzymatic hydrolysis on SSO

Glucose Yield from Enzymatic Hydrolysis - HPLC Results

Date: June 07, 2013

Table 8: Glucose standards

Standards	Concentration (g/L)	Glucose Area (uV.S)
S1	20	25526794.40
S2	5	5483267.48
S3	2	2208890.80
S4	1	1302174.20

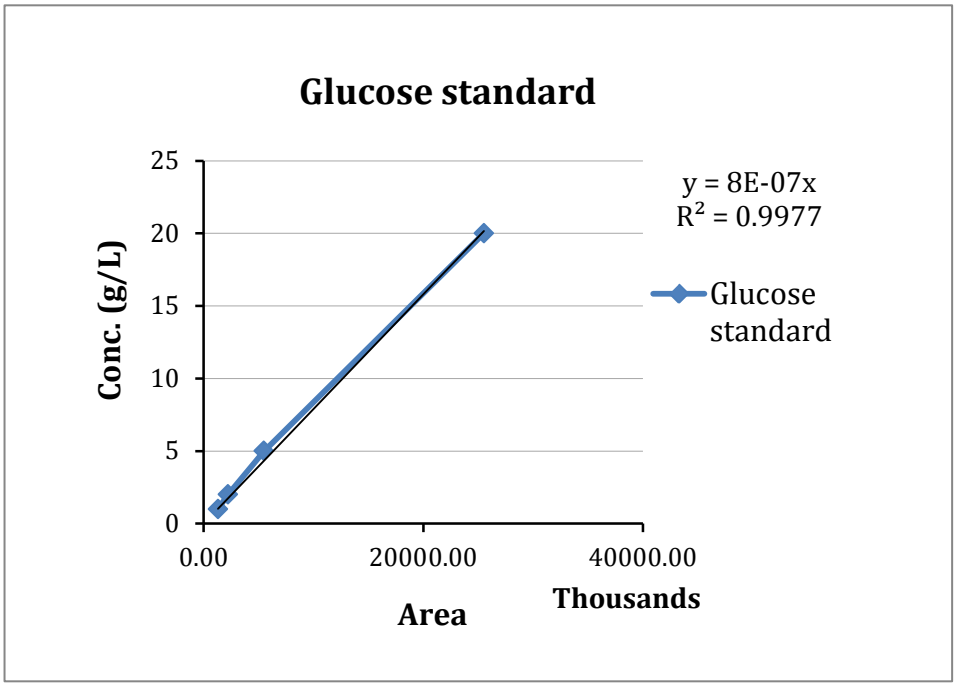


Figure 4: Glucose standards plot

Table 9: HPLC data from enzymatic hydrolysis test

Bottle Number	Sample Name	Time (h)	Glucose (HPLC)	Glucose (HPLC)	Average* ± Std. Dev. (g/L)	Glucose Yield** (%)	Corrected *** Glucose Yield (%)
			Area(µV.S)	Conc. (g/L)			
1	EH0-1	0	10392161.96	9.35	9.28	42.18	67.38
2	EH0-2		9593459.50	8.63	± 0.39		
3	EH0-3		10484796.95	9.44			
4	EH0-4		10375601.71	9.34			
5	EH0-5		10141384.28	9.13			
6	EH0-6		10872933.64	9.79			
1	EH12-1	12	9189055.08	8.27	8.33	37.86	60.49
2	EH12-2		9579356.30	8.62	± 0.69		
3	EH12-3		8979810.23	8.08			
4	EH12-4		8953359.26	8.06			
5	EH12-5		8866237.90	7.98			
6	EH12-6		9966604.55	8.97			
1	EH24-1	24	7765087.75	6.99	6.82	30.98	49.50
2	EH24-2		8111969.32	7.30	± 0.82		
3	EH24-3		6810284.52	6.13			
4	EH24-4		7086792.14	6.38			
5	EH24-5		7469906.85	6.72			
6	EH24-6		8197735.36	7.38			
1	EH48-1	48	1357805.17	1.22	1.57	7.15	11.43
2	EH48-2		1763737.14	1.59	± 0.79		
3	EH48-3		1977832.06	1.78			
4	EH48-4		326430.38	0.29			
5	EH48-5		1519931.15	1.37			
6	EH48-6		3544895.30	3.19			

Fermentation on SSO

Date: May 07, 2013

Sugar content for each 5g sample: 20 g/L Glucan (or 22 g/L Glucose)

Table 10: Ethanol standard data

Standards	Concentration (mM)	Glucose Area (uV.S)	
S1	1	n/a	n/a
S1-1		n/a	
S2	2	120340.88	119125.88
S2-1		117910.88	
S3	5	330331.42	301351.15
S3-1		272370.87	
S4	10	600482.72	578400.96
S4-		556319.20	
S5	20	1280233.40	1172104.10
S5-1		1063974.80	
S6	50	3276946.95	3126817.87
S6-1		2976688.78	

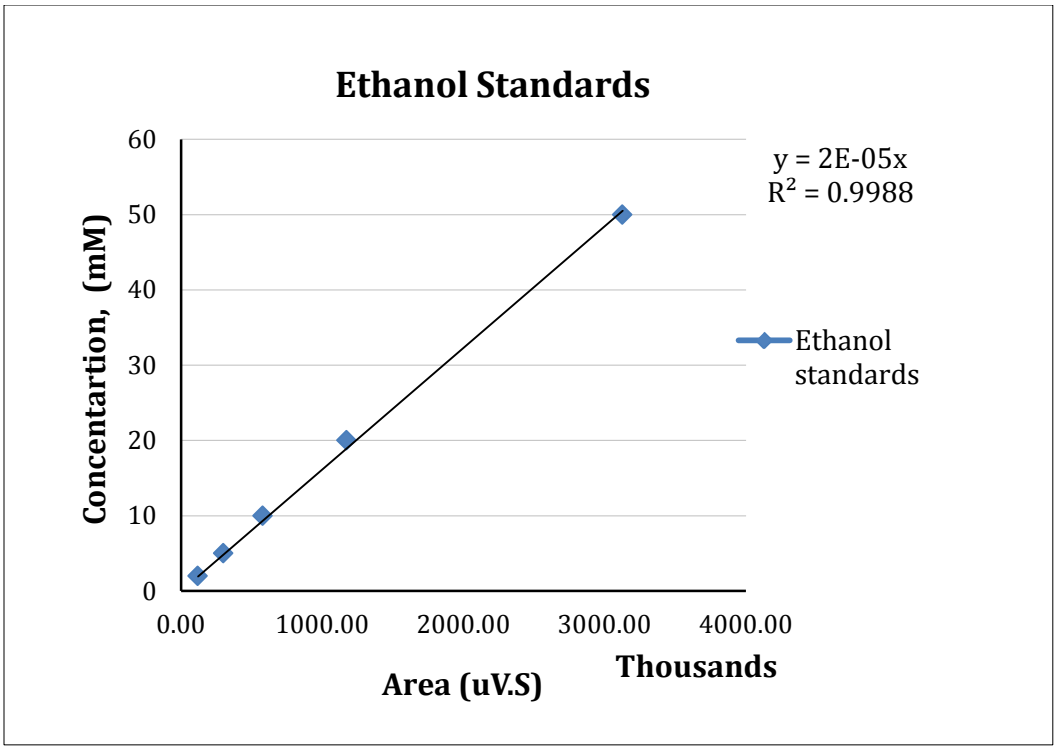


Figure 5: Ethanol standards plot

Date: May 07, 2013

Table 11: Ethanol yield data from fermentation test

Bottle Number	Sample Name	Time (hr)	Ethanol (HPLC)	Ethanol (HPLC)	Ethanol (HPLC)	Average* Ethanol Conc. ± Std. Dev. (g/L)	Corrected** Average Ethanol Conc. (g/L)	Ethanol Yield*** (%)
			Area(µV.S)	Con. (mM)	Con. (g/L)			
1	FE0-1	0	37016888.42	740.34	34.11	24.27	38.77	96.5
3	FE0-2		29498528.60	589.97	27.18	± 3.80		
5	FE0-3		12496254.80	249.93	11.51			
1	FE12-1	12	29687795.95	593.76	27.35	23.15	36.99	92.1
3	FE12-2		31453122.00	629.06	28.98	± 5.97		
5	FE12-3		14246734.09	284.93	13.13			
1	FE24-1	24	32356585.63	647.13	29.81	27.83	44.46	110.6
2	FE24-2		31799065.63	635.98	29.30	± 5.51		
3	FE24-3		26453535.85	529.07	24.37			
1	FE48-1	48	31357550.84	627.15	28.89	33.32	53.23	132.5
2	FE48-2		31582077.30	631.64	29.10	± 6.87		
3	FE48-3		45546744.41	910.93	41.97			

***Use % theoretical ethanol yield equation: where f=(3.64g/3.64g) and biomass=(3.64g/0.082L)
1000 g or 1 Kg biomass = [(33.32 g/L x 0.082 L) x 1000 g]/5 g = 546.448 g Ethanol

Date: June 06, 2013

Table 12: Ethanol yield data from fermentation test

Bottle Number	Sample Name	Time (hr)	Ethanol (HPLC)	Ethanol (HPLC)	Ethanol (HPLC)	Average* Ethanol Conc. ± Std. Dev. (g/L)	Ethanol Yield*** (%)
			Area(µV.S)	Con. (mM)	Con. (g/L)		
1	FE0-1	0	30032187.67	600.64	27.67	25.58 ± 3.80	101.7
2	FE0-2		27048584.91	540.97	24.92		
3	FE0-3		20197919.53	403.96	18.61		
4	FE0-4		29424450.76	588.49	27.11		
5	FE0-5		37060698.61	741.21	34.15		
6	FE0-6		22812194.61	456.24	21.02		
1	FE12-1	12	30922998.22	618.46	28.49	31.41 ± 5.97	124.9
2	FE12-2		32820006.21	656.40	30.24		
3	FE12-3		23201800.63	464.04	21.38		
4	FE12-4		34984526.25	699.69	32.23		
5	FE12-5		37503156.11	750.06	34.56		
6	FE12-6		45102577.78	902.05	41.56		
1	FE24-1	24	32922225.82	658.44	30.33	32.23 ± 5.51	128.1
2	FE24-2		32456699.35	649.13	29.91		
3	FE24-3		23375866.14	467.52	21.54		
4	FE24-4		36559923.72	731.20	33.69		
5	FE24-5		39232248.16	784.64	36.15		
6	FE24-6		45318342.53	906.37	41.76		
1	FE48-1	48	33503603.93	670.07	30.87	32.81 ± 6.87	130.5
2	FE48-2		33205940.41	664.12	30.60		
3	FE48-3		25545845.94	510.92	23.54		

Bottle Number	Sample Name	Time (hr)	Ethanol (HPLC)	Ethanol (HPLC)	Ethanol (HPLC)	Average* Ethanol Conc. ± Std. Dev. (g/L)	Ethanol Yield*** (%)
			Area(µV.S)	Con. (mM)	Con. (g/L)		
4	FE48-4		36075988.81	721.52	33.24		
5	FE48-5		39949968.81	799.00	36.81		
6	FE48-6		45380957.21	907.62	41.81		

Date: June 13, 2013

Table 13: Ethanol yield data from fermentation test

Bottle Number	Sample Name	Time (h)	Ethanol (HPLC) Area(μV.S)	Ethanol (HPLC) Con. (mM)	Ethanol (HPLC) Con. (g/L)	Average* Ethanol Conc. ± Std. Dev. (g/L)	Ethanol Yield*** (%)
1	FE0-1	0	25008355.52	500.17	23.04	20.20 ± 3.80	80.3
2	FE0-2		22366405.21	447.33	20.61		
3	FE0-3		22679645.00	453.59	20.90		
4	FE0-4		19456425.36	389.13	17.93		
5	FE0-5		17194847.38	343.90	15.84		
6	FE0-6		26464365.01	529.29	24.38		
7	FE0-7		28536034.18	570.72	26.29		
8	FE0-8		21376668.65	427.53	19.70		
9	FE0-9		10316929.62	206.34	9.51		
10	FE0-10		25798750.81	515.98	23.77		
1	FE12-1	12	29222315.21	584.45	26.93	27.10 ± 5.97	107.7
2	FE12-2		28481435.81	569.63	26.24		
3	FE12-3		28734987.61	574.70	26.48		
4	FE12-4		26886343.45	537.73	24.77		
5	FE12-5		27505012.56	550.10	25.34		
6	FE12-6		29881089.72	597.62	27.53		
7	FE12-7		30084484.41	601.69	27.72		
8	FE12-8		35646373.16	712.93	32.84		
9	FE12-9		26137263.62	522.75	24.08		
10	FE12-10		31493054.91	629.86	29.02		
1	FE24-1	24	33397917.61	667.96	30.77	29.27	116.4

Bottle Number	Sample Name	Time (h)	Ethanol (HPLC)	Ethanol (HPLC)	Ethanol (HPLC)	Average* Ethanol Conc. ± Std. Dev. (g/L)	Ethanol Yield*** (%)
			Area(µV.S)	Con. (mM)	Con. (g/L)		
2	FE24-2		30184427.61	603.69	27.81	± 5.51	
3	FE24-3		30290380.84	605.81	27.91		
4	FE24-4		32953400.41	659.07	30.36		
5	FE24-5		33919696.24	678.39	31.25		
6	FE24-6		31896572.89	637.93	29.39		
7	FE24-7		30901551.58	618.03	28.47		
8	FE24-8		35279318.05	705.59	32.51		
9	FE24-9		27556285.79	551.13	25.39		
10	FE24-10		31339491.77	626.79	28.88		
1	FE48-1	48	35013714.81	700.27	32.26	30.01 ± 6.87	119.3
2	FE48-2		30918388.42	618.37	28.49		
3	FE48-3		30449180.17	608.98	28.06		
4	FE48-4		33190145.61	663.80	30.58		
5	FE48-5		34721738.49	694.43	31.99		
6	FE48-6		31344345.17	626.89	28.88		
7	FE48-7		31024674.79	620.49	28.59		
8	FE48-8		36780882.21	735.62	33.89		
9	FE48-9		30560026.81	611.20	28.16		
10	FE48-10		31658412.46	633.17	29.17		

Picture 1: Dried SSO sample

Picture 2: SSO samples in the stove-oven

Picture 3: Centrifuge Sorvall RC 5C

Picture 4: Biohood

Picture 5: Cellulase activity test

Picture 6: Chemicals used for tests

Picture 7: COSLIF pretreatment phase

Picture 8: HPLC apparatus

APPENDIX B: ADSORPTION CAPACITY CALCULATIONS

Adsorption capacity constant (Langmuir Isotherms)

Date: May 17, 2014

Table 1: Data parameters from experiment

Samples	Actual Reading	Absorbance	E_{FREE}	Adsorbed	% Adsorp
Wat. Blank	0				
Subs.					
Blank	178	0.399			
E_{INIT}	395	0.859			
1	288.3	0.635	110.3	284.7	72.07595
2	293.3	0.646	115.3	279.7	70.81013
3	282.9	0.623	104.9	290.1	73.44304
4	295.1	0.65	117.1	277.9	70.35443
5	293.3	0.646	115.3	279.7	70.81013
Avg.				282.42	71.49873
Std. Dev.					1.26207

Table 2: Data parameters calculation

Actual Reading	E_{Ads}	E_{Free}	E_{ads}/E_{free}	Ee	E_{free}/Ee
282.9	290.1	104.9	2.765491	14.505	7.231989
288.3	284.7	110.3	2.581142	14.235	7.748507
293.3	279.7	115.3	2.425846	13.985	8.244548
293.3	279.7	115.3	2.425846	13.985	8.244548
295.1	277.9	117.1	2.373185	13.895	8.427492

Amount of substrate in each test sample = 100 mg, SSO waste hydrolyzed

Wat.Blank = water blank

Subs.Blank = substrate blank

E. Blank = Enzyme blank

Eads, m = maximum Accellerase 1500 adsorbed (µg of cellulases/mg of substrate)

Kads = adsorption constant $(\mu g/ml)^{-1}$

Ee = Cellulases adsorbed, µg Accellerase/ mg of substrate

Eads, m and *Kads* are obtained from plot of the following Equation:

$$\frac{E_{in\,sol,ads}}{E_e} = \frac{1}{K_{ads} \cdot E_{ads,m}} + \frac{1}{E_{ads}} \cdot E_{in\,sol,ads}$$

Slope: 0.0979; Intercept: -3.0448 (Figure 1 below)

1/intercept: 0.328428797; K_{Ads} = 0.003215318

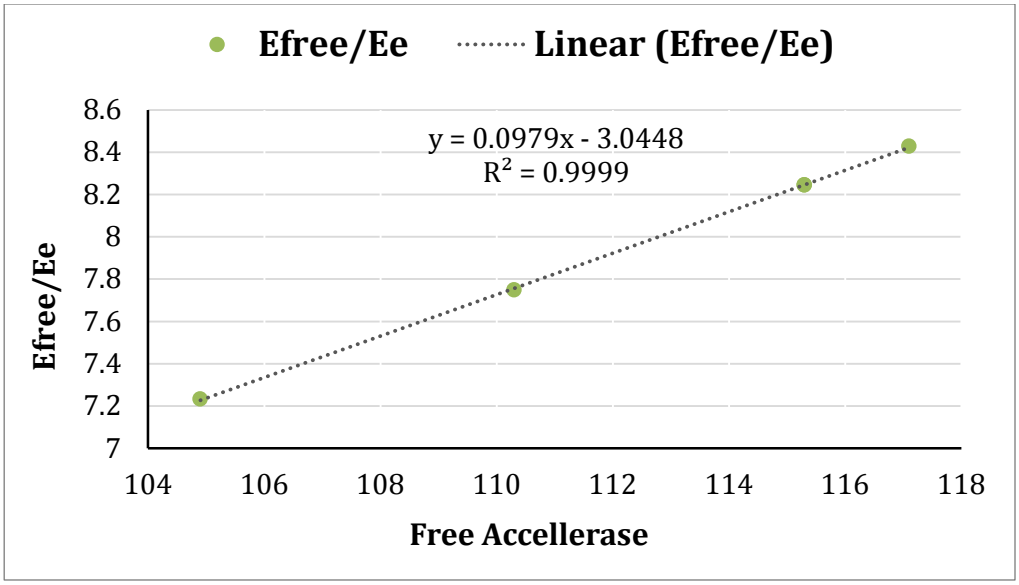


Figure 1: Langmuir isotherms plot

APPENDIX C: BERKELEY MADONNA COMPUTER PROGRAM CODE AND TABULATED DATA

Computer Program Codes and run for Kinetic Model on SSO

```
METHOD RK4
STARTTIME = 0
STOPTIME=100
DT = 0.005
DTOUT=1

GlucanInit[1]=540*0.3*0.31
XylanInit[1]=540*0.15*0.19
GlucanInit[2]=220*0.3*0.31/1
XylanInit[2]=220*0.15*0.19/1
initGlucan[1..n] = Glucan0[i]
initXylan[1..n] = Xylan0[i]
initCello[1..n] =0
initGlu[1..n] = 0
initXyl[1..n] = 0
initXc[1] = 0.3
initXc[2]=1.0
initEth[1..n] =20
initEth[1] = 20
initEth[2] = 20

Activefraction=0.90
Glucan0[1..n]=GlucanInit[i]*Activefraction
Xylan0[1..n]= Activefraction*XylanInit[i]
E_load[1]=10
E_load[2]=13
Et[1..n]=E_load[i]*Glucaninit[i]/457.1
BG[1..n]=6*Et[i]*457.1/19000
d/dt(Glucan[1..n]) = R_Glucan[i]
d/dt(Xylan[1..n]) = R_Xylan[i]
d/dt(Glu[1..n]) = R_Glu[i]
d/dt(Xyl[1..n]) = R_Xyl[i]
d/dt(Cello[1..n]) = R_Cello[i]
d/dt(Xc[1..n]) = R_Xc[i]
d/dt(Eth[1..n]) = R_Eth[i]

R_Glucan[1..n] = -(k*(1-x1[i])^m+c)*(CE[i]/Cs[i])*(Ksc/(Ksc+Cello[i]))*(Ksp/(Ksp+Eth[i]))
R_Xylan[1..n] = XylanInit[i]/GlucanInit[i]*a*h1*X1[i]^(h1-1)*R_Glucan[i]
```

R_Glu[1..n] =(-1.056*R_Glucan[i]-R_cello[i])*1.053 - (u1[i]/YXGlumax+m1)*Xc[i]
R_Xyl[1..n] = (-1.136*R_Xylan[i])-(u2[i]/YXXylmax+m2)*Xc[i]
R_Cello[1..n] = -1.056*R_Glucan[i]-Kc*Cello[i]*BG[i]/(Km*(1+Glu[i]/Kcg)+Cello[i])
R_Xc[1..n] = (u1[i]+u2[i]-kd-kde[i])*Xc[i]
R_Eth[1..n] = (u1[i]/YXGlumax+m1)*Xc[i] *YEthGlu+(u2[i]/YXXylmax+m2)*Xc[i]
*YEthXyl
kde[1..n]=a1*exp(Cae/R*Eth[i])
u1[1..n]=Glu[i]/(kGlu+Glu[i]+I1*Xyl[i])*U1eth[i]
U1eth[1..n]=0.32*(1-Eth[i]/Ethmax)^f1
u2[1..n]=if U2eth[i]>0 then U2eth[i]*(Xyl[i]-XylT)/(kXyl*Xc[i]+Xyl[i]+I2*Glu[i]) else 0
U2eth[1..n]=Umax2*(1-Eth[i]/ETHMAX2)
XylT=kXyl*m2*YXXylmax/umax2
kd=m1*YXGlumax+m2*YXXylmax
x1[1..n]=(Glucan0[i]-Glucan[i])/GlucanInit[i]
x2[1..n]=(Xylan0[i]-Xylan[i])/XylanInit[i]

GUESS E1=0.1
ROOTS E1=Et[1]-E1-(Cs[1]-1)*Ks*E1*(Glucan[1]+Xylan[1])/(1+Ks*E1)
LIMIT E1 >= 0
LIMIT E1<=Et[1]
E[1]=E1
GUESS E2=0.1
ROOTS E2=Et[2]-E2-(Cs[2]-1)*Ks*E2*(Glucan[2]+Xylan[2])/(1+Ks*E2)
LIMIT E2 >= 0
LIMIT E2<=Et[2]
E[2]=E2
Cs[1..n]=Cs0
CE[1..n]=Ks*Cs[i]*E[i]*(Glucan[i]+Xylan[i])/(1+Ks*E[i])
m1=0.034
m2=0.025
umax1=0.32; {maximum growth rate from glucose, / h}

umax2=0.2; {maximum growth rate from xylose, / h}
YXGlumax=0.08
YXXylmax=0.074
a1=0.026; {Thermal ethanol death toxin coefficient, /h}
Cae=0.0037*8.314; {Lipid-buffer partition coefficient,}
R=8.314;
Ethmax=87.8
ETHMAX2=62.3
f1=2.93; {ethanol inhibition factor to glucose consumption}
YEthGlu=0.5; {ethanol tolerance for growth in glucose, g/g}
YEthXyl=0.46; {ethanol tolerance for growth in glucose, g/g}
KGlu=0.091; {Monod growth for glucose, g/L}
KXyl=3.77; {growth constant for xylose, dimensionless}
I1=0.108; {Monod growth inhibitor from xylose, g/L}

```
I2=6.032; {Monod growth inhibitor from glucose, g/L}
n=2; {number sets of data}
a=1
h1=1;{xylan and glucan correlation constant, dimensionless}

{Hydrolysis kinetic parameters}
k=0.69; {Hydrolysis rate constant, /h}
m=0.537; {Exponent of the declining substrate reactivity, dimensionless}
c=0.00; {Conversion independent component in rate function, /h}
Ksc=5.85; {Inhibition of cellulose hydrolysis by cellibiose, g/L}
Ksp=50.35; {inhibition of cellulose hydrolysis by ethanol, g/L}
Cs0=1.29;
Cl=1.0123
Ks=0.466; {Adsorption constant for cellulosic fraction of biomass, I/U}
Kl=0.807
kf1=1.8366
kr1=kf1/Ks
kf2=0.8359
kr2=kf2/Kl
Kc=663.4
Km=10.56
Kcg=0.62; {Hydrolysis kinetic parameters from Xiongjun}
xc1=xc[1]
eth1=Eth[1]

Glu1=Glu[1]
Xyl1=Xyl[1]
Cello1=Cello[1]
xc2=xc[2]
eth2=Eth[2]
Glu2=Glu[2]
Xyl2=Xyl[2]
Cello2=Cello[2]
```

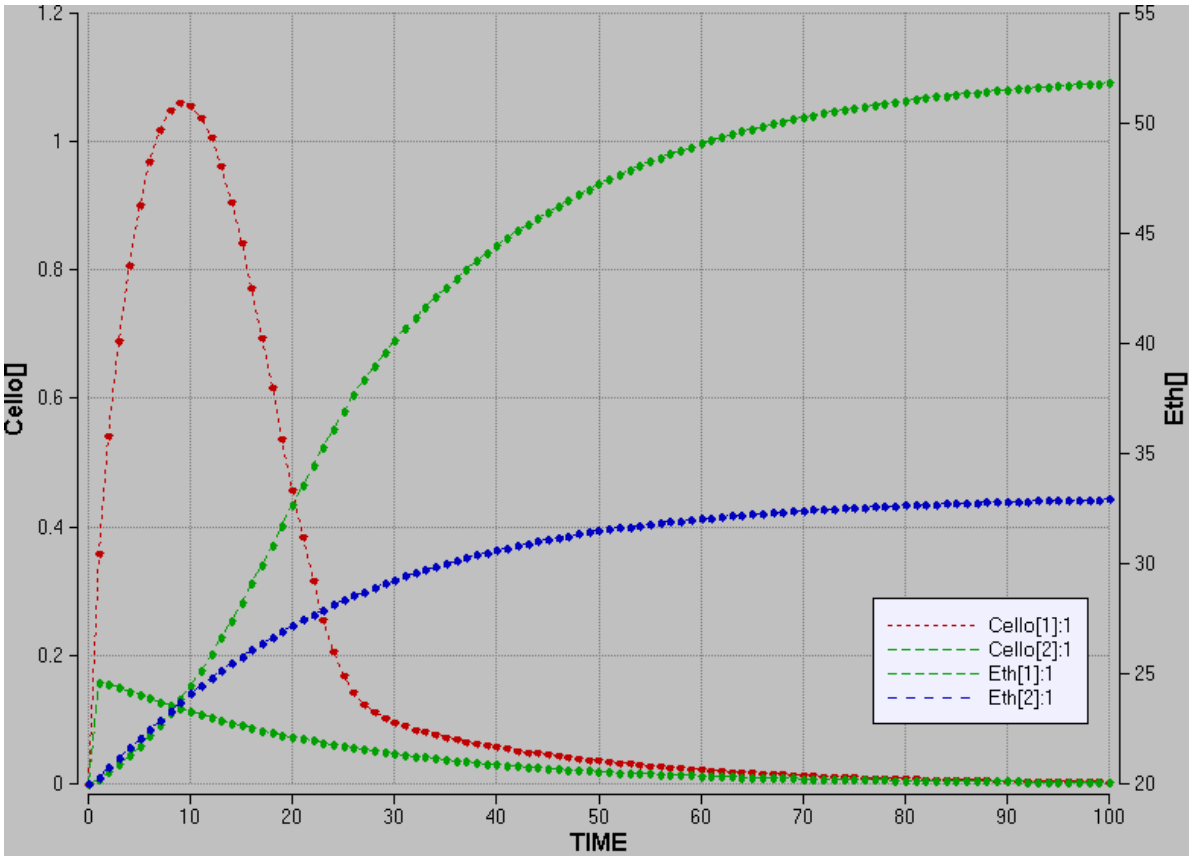


Figure 1: Kinetic model profiles (Cellomass vs Ethanol)

Table 1: Berkeley Madonna program window parameters

STARTTIME	= 0
STOPTIME	= 100
DT	= 0.005
DTOUT	= 1
ROOTTOL	= 0.001
Activefraction	= 0.9
m1	= 0.034
m2	= 0.025
umax1	= 0.32
umax2	= 0.2
YXGlumax	= 0.08
YXXylmax	= 0.074
a1	= 0.026
Cae	= 0.0307618
R	= 8.314
Ethmax	= 87.8
ETHMAX2	= 62.3
f1	= 2.93
YEthGlu	= 0.5
YEthXyl	= 0.46
KGlu	= 0.091
KXyl	= 3.77
I1	= 0.108
I2	= 6.032
n	= 2
a	= 1
h1	= 1
k	= 0.69
m	= 0.537
c	= 0
Ksc	= 5.85
Ksp	= 50.35
Cs0	= 1.29
Cl	= 1.0123
Ks	= 0.466
Kl	= 0.807
kf1	= 1.8366
kf2	= 0.8359
Kc	= 663.4

Table 2: Tabulated data from computer runs (Cellomass vs Ethanol)

TIME	Cello[1]:1	Cello[2]:1	Eth[1]:1	Eth[2]:1
0	0	0	20	20
1	0.359837	0.156938	20.2244	20.3072
2	0.542771	0.155468	20.5344	20.7419
3	0.690236	0.150115	20.884	21.186
4	0.808349	0.144322	21.2718	21.6287
5	0.900882	0.138482	21.699	22.0652
6	0.970553	0.132748	22.1667	22.4926
7	1.01946	0.127197	22.6761	22.9091
8	1.04932	0.121861	23.228	23.3135
9	1.06161	0.116749	23.8226	23.7052
10	1.0577	0.111861	24.4598	24.0839
11	1.03891	0.107187	25.1386	24.4494
12	1.00659	0.102717	25.8574	24.802
13	0.962152	0.098439	26.6141	25.1417
14	0.907135	0.094342	27.4057	25.4689
15	0.843202	0.090415	28.2286	25.7837
16	0.772169	0.086649	29.0786	26.0866
17	0.696002	0.083035	29.9509	26.3778
18	0.616815	0.079565	30.84	26.6578
19	0.536847	0.076232	31.7401	26.9268
20	0.458439	0.073031	32.6441	27.1852
21	0.383996	0.069954	33.5443	27.4334
22	0.315919	0.066997	34.4318	27.6717
23	0.256472	0.064156	35.2961	27.9004
24	0.207499	0.061426	36.1259	28.1199
25	0.16991	0.058803	36.9105	28.3304
26	0.14311	0.056283	37.6437	28.5323
27	0.124945	0.053863	38.3256	28.7259
28	0.112593	0.051539	38.9615	28.9115
29	0.10367	0.049309	39.5584	29.0893
30	0.096677	0.047169	40.1217	29.2597
31	0.090822	0.045116	40.6552	29.423
32	0.085714	0.043148	41.1617	29.5793
33	0.08115	0.041261	41.6434	29.729
34	0.077011	0.039454	42.102	29.8724
35	0.073218	0.037722	42.5393	30.0096
36	0.069715	0.036064	42.9567	30.1409
37	0.066456	0.034477	43.3554	30.2666
38	0.063405	0.032958	43.7366	30.3869
39	0.060536	0.031506	44.1014	30.5019
40	0.057824	0.030117	44.4507	30.612
41	0.055253	0.02879	44.7854	30.7174

TIME	Cello[1]:1	Cello[2]:1	Eth[1]:1	Eth[2]:1
42	0.052807	0.027521	45.1062	30.8181
43	0.050474	0.026309	45.4139	30.9145
44	0.048245	0.025151	45.7089	31.0067
45	0.046113	0.024045	45.992	31.0949
46	0.044069	0.02299	46.2635	31.1792
47	0.04211	0.021982	46.5241	31.2599
48	0.040231	0.02102	46.7742	31.3371
49	0.038428	0.020103	47.0141	31.411
50	0.036698	0.019227	47.2442	31.4816
51	0.035037	0.018392	47.465	31.5492
52	0.033444	0.017594	47.6768	31.6139
53	0.031916	0.016834	47.8799	31.6758
54	0.030452	0.016109	48.0747	31.735
55	0.029048	0.015417	48.2615	31.7917
56	0.027703	0.014757	48.4404	31.846
57	0.026416	0.014127	48.612	31.8979
58	0.025185	0.013527	48.7763	31.9477
59	0.024007	0.012954	48.9338	31.9953
60	0.022881	0.012407	49.0845	32.0409
61	0.021805	0.011885	49.2289	32.0846
62	0.020778	0.011388	49.3672	32.1265
63	0.019797	0.010913	49.4995	32.1666
64	0.018862	0.010459	49.6261	32.2051
65	0.017969	0.010026	49.7473	32.2419
66	0.017119	0.009613	49.8632	32.2772
67	0.016308	0.009219	49.9741	32.3111
68	0.015536	0.008842	50.0801	32.3435
69	0.0148	0.008482	50.1815	32.3747
70	0.014099	0.008138	50.2785	32.4045
71	0.013432	0.00781	50.3711	32.4332
72	0.012797	0.007496	50.4597	32.4607
73	0.012193	0.007196	50.5444	32.4871
74	0.011618	0.006909	50.6253	32.5124
75	0.011071	0.006634	50.7026	32.5367
76	0.01055	0.006372	50.7765	32.5601
77	0.010054	0.006121	50.8471	32.5825
78	0.009583	0.005881	50.9145	32.6041
79	0.009135	0.005651	50.979	32.6248
80	0.008708	0.005431	51.0405	32.6446
81	0.008302	0.005221	51.0993	32.6637
82	0.007915	0.005019	51.1554	32.6821
83	0.007548	0.004826	51.2091	32.6998
84	0.007198	0.004641	51.2603	32.7167

TIME	Cello[1]:1	Cello[2]:1	Eth[1]:1	Eth[2]:1
85	0.006865	0.004464	51.3092	32.733
86	0.006548	0.004294	51.3559	32.7487
87	0.006246	0.004131	51.4005	32.7638
88	0.005959	0.003975	51.4431	32.7783
89	0.005686	0.003825	51.4838	32.7923
90	0.005426	0.003682	51.5227	32.8057
91	0.005178	0.003544	51.5597	32.8187
92	0.004942	0.003412	51.5952	32.8311
93	0.004717	0.003285	51.629	32.8431
94	0.004503	0.003164	51.6613	32.8546
95	0.004299	0.003047	51.6922	32.8657
96	0.004104	0.002935	51.7216	32.8764
97	0.003919	0.002827	51.7498	32.8867
98	0.003742	0.002723	51.7766	32.8967
99	0.003574	0.002624	51.8023	32.9062
100	0.003413	0.002528	51.8268	32.9154

APPENDIX D: EXPERIMENTAL PROCEDURES

1. Quantitative saccharification (QS)

Materials/ Equipment Required:

- Sugars, high purity for standards (98% +) – glucose, xylose, galactose, arabinose, mannose for examples
- H_2SO_4,72% w/w (12.00 ± 0.02 M or specific gravity 1.6389 at 15.6°C) - (RICCA R8191600-4A)
- Calcium carbonate, ACS grade (Fisher C64-500)
- DDW
- Glass stirring rod, 6”
- Serum glass bottles, crimp top style 125 mL
- Crimp cap, aluminum (Fisher 0640614B)
- Rubber stoppers, blue (Fisher FSSP9717931)
- Crimper
- pH paper for pH range between 0 and 14
- Syringe, sterile 3 mL (for sampling) - (Fisher B309657)
- Needle, sterile 23Gx1.5 (for sampling) – (Fisher 14-826-6C)
- Syringe filter, 0.22 um (for sampling) – (VWR CA28145-491)
- Microcentrifuge tubes, 2 mL (for sampling) – (Fisher 05-408-138)
- Autosampler vials with cap, 2 mL (for HPLC) - (Fisher 03378397)
- Pipette, disposable 10 mL (for pH adjustment) – (Fisher 07-200-574)
- Pipette pump

- Erlenmeyer flasks, 50 mL
- Convection ovens with temperature control to 45 ± 3°C and 105 ± 3°C
- Autoclave capable of maintaining 121 ± 3°C (Yamato SM300)
- Water bath set at 30 ± 1°C (Grant OLS200)
- Benchtop centrifuge (for HPLC sample preparation) – (Eppendorf 5424)
- High Performance Liquid Chromatography (HPLC)-Perkin Elmer LC Autosampler, Series 200
- HPLC column, Bio-Rad Aminex7 HPX-87P
- Guard columns, cartridges appropriate for the column used
    - Deashing guard column cartridges (#125-0118), of the ionic form H^+/CO_3 from Bio-Rad, in series with cartridge holders (#125-0131).

Procedure:

1. Oven dried sample at 45^{o}C.
2. Ensure particles of sample pass through 40 mesh screen by breaking up the solids during the drying process.
3. Place 0.3 ± 0.01 g (W_1) of sample in 125 mL glass serum bottle (each sample must be run at least in duplicate).
4. Add 3.0 ± 0.01 mL of 72% H_2SO_4.
5. Mix for 1 minute with a glass stirring rod until sample is thoroughly wetted.
6. Place glass serum bottle in water bath at 30 ± 1°C and hydrolyze for 1 hour.
7. Stir sample every 15 minutes to assure complete mixing and wetting.

8. Dilute to a 4% acid concentration by adding 84.00 ± 0.04 mL DDW. This can be done by using a scale.
9. Prepare sugar recovery standards (SRS) by placing 0.3 g of high purity sugar of interest (pre-dried at 45^{o}C) to the nearest 0.1 mg into a 125 mL glass serum bottle (in duplicate). Add 10 mL of DDW and then add 348 uL of 72% H_2SO_4.
    - The calculated sugar recovery standards (SRS) will be used to correct for losses due to the destruction of sugars during the hydrolysis process.
    - % SRS = $\frac{conc.\ detected by HPLC, \frac{mg}{mL}}{known conc.\ of sugar before hydrolysis, \frac{mg}{mL}}$ x 100
10. Stopper and crimp each bottle.
11. Mix the samples by inverting the serum bottles a few times to eliminate phase separation.
12. Autoclave the bottles for 1 hour at 121 ± 3^{o}C (liquid cycle only).
13. Allow bottles to cool for 20 minutes at room temperature before removing the seals and stoppers.
14. Transfer 20 mL supernatant of each hydrozate to 50 mL Erlenmeyer flasks.
15. Neutralize each flask to between pH 5 and pH 6 by adding calcium carbonate slowly with frequent swirling to avoid foaming. Check pH with pH paper to avoid over neutralization.
16. Filter each neutralized hydrolyzate using 3 mL syringe with 0.2 um filter. One portion goes into a Safe-Lock 1.5 mL microcentrifuge tube for fridge storage in case a repeat analysis is required (no more than 2 weeks in storage); the other portion goes directly into autosampler vial if dilution is not required. Dilution is required when the concentration is out of the calibration standard range.

17. Prepare sugar calibration standards for each sugar of interest or a set of multi-component standards containing glucose, xylose, arabinose in the range of 0.2 to 12.0 mg/mL using HPX-87P column.
18. Instrumental conditions for HPX-87P column:
    - Sample volume: 50uL.
    - Eluant: 0.2 um filtered and degassed, deionized water.
    - Flow rate: 0.6 mL/min.
    - Column temperature: 85^{o}C.
    - Detector: refractive index.
    - Run time: 15 minutes data collection plus a 75 minute pump ramp.
        - Note: Check test sample chromatograms. Levels of cellobiose greater than 3 mg/ mL indicate incomplete hydrolysis and peaks appear before cellobiose may indicate sugar degradations.
        - Details of procedure for HPLC sample preparation are in the fermentation section.

2. Cellulase activities

Cellulase activity is measured in terms of Filter Paper Units (FPU) per milliliter of original (undiluted) enzyme solution. The value of 2.0 mg of reducing sugar as glucose from 50 mg of filter paper (4% conversion) in 60 minutes has been designated as the intercept for calculating Filter Paper cellulase Units (FPU).

Materials/ Equipment Required:

- DDW (distilled de-ionized water)
- 2.65 g – 3,5 Dinitrosalicylic acid (Sigma 128848-100G)
- 4.95 g – Sodium hydroxide (Fisher S318-500)
- 76.5 g – Rochelle salts (sodium potassium tartrate) – (Fisher S387-500)
- 1.9 mL – Liquified Phenol (melt at 50°C) – (Sigma P9346-500ML)
- 2.075 g – Sodium metabisulfite (Sigma S9000-500G)
- 210 g – Citrate acid monohydrate (Fisher A104-500)
- 0.1 N HCl (Fisher 351280-500)
- Anhydrous glucose (Sigma G8270-1KG)
- Accellerase 1500
- Whatman No. 1 filter paper
- 5 – Test tube, plastic 50 mL (for enzyme dilution) - (VWR 82018-050)
- 30 – Test tube, plastic 15 mL (26 for samples and 4 for glucose dilution) - (VWR 21008-216)
- Test tube racks
- 26 – Cuvette w/ Cap (VWR 97000-584 and 89000-628)
- 1 – Graduated cylinder, 50 mL (for 1:20 enzyme dilution)
- 1 – Beaker, 250 mL (for DNS reagent)
- 1 – Beaker, 1 L (for Citrate buffer)
- 2 – Volumetric flask, 100 mL (for citrate buffer and glucose stock solution)
- Hot plate

- Water bath capable of maintaining $50^{o}C \pm 0.1^{o}C$ (Grant OLS200)
- Spectrophotometer suitable for measuring absorbance at 540 nm

Procedure for DNS reagent:

1. Mix and dissolve:
    - 1416 mL – DDW
    - 10.6 g – 3,5 Dinitrosalicylic acid
    - 19.8 g – Sodium hydroxide
2. Add:
    - 306 g – Rochelle salts (sodium potassium tartrate)
    - 7.6 mL – Phenol (melt at $50^{O}C$)
    - 8.3 g – Sodium metabisulfite
3. Take 3 ml from the above mix and titrate with 0.1 N HCl to the phenolphthalein endpoint. It should take 5-6 mL of HCl.
4. Add NaOH to the mix if it takes less than 5-6 ml of HCl. No need to add if it is more (2g = 1 mL 0.1 N HCl).

Note:

- Make enough DNS reagent to be used. Avoid large batch that requires storage because of oxidation of sodium sulfite (Miller, 1958).
    - Example (use only 1/10 of the above amounts):
        - 141.6 mL – DDW
        - 1.06 g – 3,5 Dinitrosalicylic acid

- 1.98 g – Sodium hydroxide
- 30.6 g – Rochelle salts (sodium potassium tartrate)
- 0.76 mL – Phenol
- 0.83 g – Sodium metabisulfite

Procedure for 1 M Citrate Buffer:

1. Mix and dissolve in 100 mL volumetric flask to make 1 M stock (mix Citric acid to DDW first before adding NaOH to avoid violent chemical reaction):
    - 21 g – Citric acid monohydrate
    - 75 mL – DDW
    - 5 to 6 g – NaOH (add until pH = 3)
2. Dilute to 100 mL and check pH
3. Add NaOH until pH = 4.5 if necessary
4. Dilute the 1 M citrate buffer stock to 0.05 M (make 1 L)
5. Check the pH (should be 4.8). Adjust the pH to 4.8 if necessary

Procedure for Filter Paper Assay (Accelerase 1500):

1. Prepare 50 mg (triplicate-15 samples) Whatman No. 1 filter paper strip (1.0 x 6.0 cm);
2. Place a rolled filter paper strip into each 15 mL test tube (plastic).
3. Add 1.0 mL 0.05 M citrate buffer, pH 4.8 to each tube; the buffer should saturate the filter paper strip.
4. Bring the tubes with their contents to 50^{o}C in water bath.

5. Add 0.5 ml enzyme diluted appropriately in citrate buffer to each tube (enzyme dilutions are made in citrate buffer from stock solution that has been diluted 1:20 in citrate buffer).
6. Incubate tubes at 50^{o}C for exactly 60 minutes.
7. Remove tubes from incubator.
8. Stop the reaction by immediately adding and mixing 3.0 mL DNS reagent to each tube.
9. The above procedure applies to blanks, controls and glucose standards (see note below).
10. Boil all tubes for exactly 5.0 minutes in boiling water bath (ensure contents in tubes are below the water level).
11. Transfer tubes to ice cold water.
12. Allow pulp to settle in each tube.
13. Dilute each tube in DDW in a cuvette (0.2 mL of color-developed reaction mixture plus 2.5 mL of DDW).
14. Mix well.
15. Determine absorbance against reagent blank at 540 nm.
16. Plot glucose standard curve. Glucose amounts (mg/0.5 mL) on x-axis vs. A_{540} on y-axis
17. Determine glucose released for each sample using the standard curve.
18. Plot glucose released (mg/0.5 mL) on x-axis versus enzyme concentration on y-axis using semi logarithmic graph paper.
19. Estimate enzyme concentration which would have release 2.0 mg of glucose by drawing a line through two data points that are closest to 2.0 mg on the x-axis. Use this line to find the enzyme concentration that would produce exactly 2.0 mg of glucose.
20. Input the estimated enzyme concentration in the following equation to find FPU/mL:

- Filter Paper Activity = $\frac{0.37}{Enzymeconcentrationreleasing\ 2.0\ mgglucose}$ units/mL
- Note: Enzyme concentration refers to the number of mL of the original solution present in each mL of the dilution.

Note:

- Reagent blank = 1.5 mL citrate buffer.
- Enzyme control = 1.0 mL citrate buffer + 0.5 mL enzyme dilution (one for each dilution).
- Substrate control = 1.5 mL citrate buffer + filter paper strip.
- Glucose standards:
  - Make up working stock of anhydrous glucose (10 mg/mL).
  - Dilutions are made as follows:

Glucose Stock (mL)	Citrate buffer (mL)	Dilution	Concentration	A_{540} (nm)
1.0	0.5	1:1.5	3.35 mg/0.5 mL	
1.0	1.0	1:2	2.50 mg/0.5 mL	
1.0	2.0	1:3	1.65 mg/ 0.5 mL	
1.0	4.0	1:5	2.00g/ 0.5 mL	

- Add 0.5 mL of each of the dilution above to 1.0 mL of citrate buffer in a 15 mL test tube (plastic).

3. Modified COSLIF pretreatment

In this study, 95% (v/v) ethanol was used as the only organic solvent in the modified COSLIF pretreatment.

Materials/ Equipment Required:

- 55 g – SSO sample (for 11 samples of 5 g each with one as substrate blank)
- 11 – Sterilized centrifuge bottle, 250 mL
- 1 – Beaker, 2 L (for supernatant)
- 1 – Graduated cylinder, 50 mL (for measuring phosphoric acid)
- 2 – Graduated cylinder, 200 mL (one for measuring ethanol and one for water)
- 440 mL – Phosphoric acid 85% (Fisher A242-1)
- 3300 mL – Ethanol 95% (Commercial Alcohols P016EA95)
- 4400 mL – DDW
- 11 - Glass stir rod, 6”
- Benchtop shaking incubator (ThermoMaxQ 4450)
- Centrifuge (Sorvall RC-5C PLUS)

Autoclave:

- 11 - Centrifuge bottle, 250 mL
- 4400 mL - DDW
- 11 - Glass stir rod, 6”

Procedure:

1. Weigh bottles and label them;
2. Place 5 grams of dry lignocellulosic sample in a 250 mL centrifuge bottle and then mix with 40 ml of 85% concentrated phosphoric acid using a glass rod;
3. Incubate the solid/liquid slurry in the MaxQ 4450 benchtop shaking incubator at 150 rpm and $50^{o}C \pm 0.2^{o}C$ for 2 hours;
4. Add 100 ml of ethanol into bottle and mix well by force to stop the biomass dissolution and weak hydrolysis reactions;
5. Centrifuge the bottle in a Sorvall RC-5C PLUS floor model centrifuge at 7000 rpm, room temperature for 15 minutes;
6. Decant the supernatant containing ethanol, acetic acid, phosphoric acid and lignin;
7. Add 200 mL of ethanol into the bottle containing the slurry of cellulose and hemicelluloses and mix well by force;
8. Repeat step 5 and step 6;
9. Add 200 mL of water into the bottle containing the solid pellet;
10. Centrifuge the bottle as in step 5;
11. Decant the supernatant containing ethanol and hemicelluloses;
12. Repeat step 9, 10 and 11 once more;
13. Weigh bottles with contents to determine the amount of water retained.

4. Enzymatic hydrolysis:

Materials/ Equipment Required:

- 10 mL - Filtered Accellerase 1500

- NH_4OH (for pH adjustment) – (EMD AX1303-6)
- HCl 1N (for pH adjustment) – (Fisher 351280-500)
- 70% ethanol spray bottle (for disinfection)
- 810 mL – Sterilized DDW
- 12 – Centrifuge bottle, 250 ml
- 1 - Graduated cylinder, 50 mL (for dilution of cellulosic pellets)
- 1 – Beaker, 250 mL (for pH meter)
- 2 – Pipette, disposable 10 mL (for pH adjustment) – (Fisher 07-200-574)
- 1 – Pipette pump
- Pipettor
- 1 box - Pipette tip, sterile 1000 uL (for sampling) – (VWR 89079-470)
- 45 – Syringe, sterile 3 mL (for sampling) - (Fisher B309657)
- 45 – Needle, sterile 23Gx1.5 (for sampling) – (Fisher 14-826-6C)
- 45 - Syringe filter, 0.22 um (for sampling) – (VWR CA28145-491)
- 45 - Microcentrifuge tubes, 2 mL (for sampling) – (Fisher 05-408-138)
- 1 – Stericup filter unit 0.22 um, 250 mL (for Accelerase 1500) – (Fisher SCGVU02RE)
- Biological hood
- pH meter
- Benchtop shaking incubator (ThermoMaxQ 4450)

Autoclave:

- 810 mL - DDW

- 1 box – Pipette tips, 1000 uL
- 50 mL graduated cylinder

Procedure - (modified for 10 samples + 1 substrate blank + 1 enzyme blank)

1. Procedure is to be carried out under a biological hood.
    - Procedure for using the biological hood:
        - Wipe the inside of the hood with 70% ethanol;
        - Place everything including samples inside the hood (to avoid contamination);
        - Turn on the UV light and the blower for 20 minutes;
        - Turn off the UV light but keep the blower on.
2. Allow samples to thaw if frozen.
3. Using the results from QS, dilute the aqueous treated biomass in the 250 mL centrifuge bottle (after ethanol and water washes) to 20 g glucan/L (including the amount of enzyme) using sterilized DDW.
    - Example:
        - Average 1.5 g of glucose is found in the 5 g SSO samples (from *QS);
        - Glucan = 0.9 x 1.5 g = 1.35 g
        - mL DDW to make 20 g/L glucan = $\frac{1.35\ g}{20\ g/L} \times 1000 = 67.5$ mL

        *QS – quantitative saccharification procedure
4. Shake well.
5. Adjust the pH value to 4.8 using NH_4OH (as a source of nitrogen).

6. Bring the contents of each bottle to 50°C (this step must be done prior to the addition of enzyme if the rate of enzymatic release of glucose is to be measured).
7. Add filtered Accellerase 1500 loading of 30 FPU per g glucan.
    - Example:
        - Filter paper unit = 53 FPU/ mL
        - Glucan = 1.35 g
        - mL to make 30 FPU/g glucan = $\frac{30\frac{FPU}{g}\times 1.35\ g}{53\ FPU/mL}$ = 0.76 mL
8. Incubate and shake sample at 50^{o}C ± 0.2^{o}C and 250 rpm for 72 hours (or until the amount of released glucose becomes negligible when measured by HPLC, Bio-Rad HPX-87P column).

If the rate of released glucose is to be measured:

1. Remove 1.5 mL aliquot from flask at 0, 12, 24, 48 and 72;
2. Expel sample into 2 mL microcentrifuge tube;
3. Centrifuge sample then filter it into autosampler vial using syringe filter 0.2um Analyze the supernatant for released (soluble) glucose using HPLC, Bio-Rad HPX-87P column).

Calculation for percent digestion:

1. Determine glucose concentration in the supernatant using HPLC, Bio-Rad HPX-87P column;

2. Subtract the glucose concentration, if any, from the substrates and enzyme blanks (see note below);
3. Correct for hydration (multiply the glucose reading by 0.9 to correct for the water molecule added in the hydrolysis of the cellulose) and multiply by the total volume of assay.
    - Example:
        - Glucose concentration in the supernatant (corrected with blanks) = 9.9 mg/mL;
        - Total volume of essay = 10 mL;
        - Then the amount of cellulose digested = 0.0099 g/mL x 10 mL x 0.9 =0.0891 g;
4. % digestion = $\frac{grams\ cellulose\ digested}{grams\ cellulose\ added}$ x 100

Note:

- Substrate blank contains DDW, NH_4OH, substrate and identical amount of volume with no enzyme.
- Enzyme blank contains DDW, NH_4OH, enzyme and identical amount of volume with no substrate.

5. Fermentation

Materials/Equipment Required:

- 2.5 g – Yeast extract (Fisher BP1422-500)
- 0.5 g – Potassium phosphate monobasic (KH_2PO_4) – (EMD PX1565-1)

- 25 g – Glucose (Sigma G8270-1KG)
- 5 g – Xylose (Fisher X1500-500G)
- NH_4OH (for pH adjustment) – (EMD AX1303-6)
- HCl 1N (for pH adjustment) – (Fisher 351280-500)
- 1 L - DDW
- 5 mL - 40% glycerol
- 70% ethanol spray bottle
- Z. mobilis 8b cell stock
- Pipettor
- 2 boxes - Pipette tip
- Pipette pump
- 3 - Pipette, disposable 10 mL (for pH adjustment and 10% transfer in fermentation procedure) - (Fisher 07-200-574)
- 1 - Graduated cylinder, 50 mL (for 10X RM rich media)
- 1 - Graduated cylinder, 250 mL (for 10:2 RMGX media)
- 1 - Volumetric flask, 250 mL (for 10:2 RMGX media)
- 1 – Glass stir rod, 8” (for mixing in rich media)
- 3 – Graduated cylinder, 100 mL (for seeding and preparing blanks)
- 1 – Beaker, 250 mL (for pH meter)
- 15 - Stericup filter unit 0.22 um, 250 mL (1 for media and 14 for hydrolysate) – (Fisher SCGVU02RE)
- 15 - Test tube, plastic 15 mL (1 for pre-seed procedure and 14 for cell harvest) - (VWR 21008-216)

- Test tube rack
- Cuvette w/ Cap (for checking OD) - (VWR 97000-584 and 89000-628)
- 15 – Serum glass bottles, crimp top style 125 mL (1 for seed procedure, 10 for hydrolysate, 1 for controlled glucose, 1 for DDW blank, 1 for substrate blank and 1 for enzyme blank)
- 15 - Crimp cap, aluminum (Fisher 0640614B)
- 15 - Rubber stoppers, blue (Fisher FSSP9717931)
- Crimper
- 10 – Cryovial 2 mL (VWR 16001-102)
- 100 - Syringe, sterile 3 mL (for sampling) - (Fisher B309657)
- 100 - Needle, sterile 23Gx1.5 (for sampling) – (Fisher 14-826-6C)
- 100 - Syringe filter, 0.22 um (for sampling) – (VWR CA28145-491)
- 100 - Microcentrifuge tubes, 2 mL (for sampling) – (Fisher 05-408-138)
- 100 - Autosampler vials with cap, 2 mL (for HPLC) - (Fisher 03378397)
- Biological hood
- Balance
- pH meter
- Vortex
- Spectrophotometer suitable for measuring absorbance at 540 nm (Eppendorf, BioPhotometer)
- Benchtop centrifuge (for cell harvest) - (Thermo Sorvall Legend RT+)
- Benchtop shaking incubator (ThermoMaxQ 4450)
- Benchtop centrifuge (for HPLC sample preparation) – (Eppendorf 5424)

- HPLC-Perkin Elmer LC Autosampler, Series 200
- HPLC column, Bio-Rad Aminex7 HPX-87P (for glucose) and Bio-Rad Aminex7 HPX-87H (for ethanol)
- Guard columns, cartridges appropriate for the column used
    - For P column, use deashing guard column cartridges (#125-0118), of the ionic form H^+/CO_3 from Bio-Rad, in series with cartridge holders (#125-0131). For H column, use Cation H cartridge (#125-0129) with holder (#125-0131).

Autoclave:

- 1 - Graduated cylinder, 50 mL (for 10X RM rich media)
- 1 - Graduated cylinder, 250 mL (for 10:2 RMGX media)
- 1 - Volumetric flask, 250 mL (for 10:2 RMGX media)
- 3 – Graduated cylinder, 100 mL (for seeding and preparing blanks)
- 1 – Beaker, 250 mL (for pH meter)
- 3 – 100 mL graduated cylinder (for seeding and preparing blanks)
- 15 - Test tube, plastic 15 mL (1 for pre-seed procedure and 14 for cell harvest) - (VWR 21008-216)
- 1 – Glass stir rod, 8” (for mixing in rich media)
- 2 boxes - Pipette tip
- 15 - Rubber stoppers, blue (Fisher FSSP9717931)
- 10 – 40% Glyserol in cryovial
- 1 L - DDW

Procedure for Rich Media (10XRM):

- Yeast Extract: 100 g/L
- Potassium Phosphate Monobasic (KH_2PO_4): 20 g/L
- DDW
- Sterilize with 0.2 um filter

Note:

- Make only 25 mL of 10X RM (add 2.5 g Yeast Extract and 0.5 g KH_2PO_4 to 50 mL DDW).
- Gradually adding Yeast Extract and KH_2PO_4 to DDW and simultaneously shaking the graduated cylinder to ensure the solutes are completely dissolved.

Procedure for 10:2 RMGX Media (revive and intermediate seed):

- 1X RM
- Glucose: 100 g/L
- Xylose: 20 g/L
- DDW
- Sterilize with 0.2 um filter

Note:

- Dilute 10X RM with DDW to make 1X RM (transfer 25 mL of 10X RM to 250 mL graduate cylinder and fill it with DDW to 250 mL mark).
- Add 25 g Glucose and 5 g Xylose to a 250 mL volumetric flask and fill it with 1X RM to marking).

Procedure for revive (pre-seed):

1. Remove the cell stock vial(s) from the freezer and allow it to gradually thaw at room temperature (at least 0.5 hour).
2. Vortex the cell stock vial(s) for 5 seconds.
3. Pipette (to prevent cells being stuck to vial wall) it into the revive media at a concentration of 10% using 15 mL centrifuge vial, that is, 1 ml of cell stock into a 9 ml of filter-sterilized (0.2 um filter) nutrient media (10:2 RMGX).
4. Incubate the culture at 33^{o}C for approximately 8 hours.
5. Take a sample under biological hood and measure OD at 600 nm:
    - Take 0.5 ml of sample in a cuvette (allow bubbles to be released);
    - Add 2.5 ml sterile DDW;
    - Mix well;
    - Read and record OD at 600 nm (adjustment for dilution is required).
6. Calculate the volume needed to inoculate seed media (10:2 RMGX) at an OD of 0.01 in the procedure for seed;
    - Example:

- Recorded OD = 4
- Required OD (for seed media) = 0.01
- Working Volume (for seed media) = 100 ml
- $C_1V_1 = C_2V_2$ => $(4)V_1 = (0.01)(100)$ => $V_1 = 0.25$ ml

Procedure for seed:

1. Transfer calculated amount (0.25 mL) of revive culture to 125 mL serum bottle with crimp top containing 100 mL of seed media (10:2 RMGX);
2. Incubate the inoculated seed bottle in the shaking incubator at 150 rpm and 33^{o}C for 12-14 hours to reach an acceptable OD and glucose concentration;
3. Harvest the cell (stop the shaking incubator) when glucose concentration of the media reaches an acceptable OD (2.0 to 3.8) as determined from cell growth curves.

Procedure for restocking cells:

1. Mix sterile DDW with glycerol to a final concentration of 40%;
   Place 500 ul of 40% glycerol solution into each cryovial;
2. Autoclave this mixture with caps on but not shut tightly;
3. When they are cooled down, add 500 ul of an overnight culture (grown in growth media) to each cryovial;
4. Vortex thoroughly and store at – 80^{o}C.

Procedure for Fermentation:

1. This procedure is to be carried out under the biological hood;
2. Create a controlled glucan (20 g/L) and blank (sterilized DDW), having the sample conditions as the hydrolysate;
3. Filter hydrolysate using 250 mL stericup;
4. Adjust pH of the hydrolysate to 6 using NH_4OH and 1N HCl;
5. Transfer the hydrolysate to 125 mL serum bottle and use a scale to determine the amount;
6. For each bottle, transfer 10% (v/v) of the seed media to a 15 mL centrifuge tube and centrifuge at 5000 rcf (or 3500 rpm) for 5 minutes at 4^{o}C. Decant the supernatant. Re-suspend the cell pellet with the hydrolysate by vortex. Transfer the volume back into the 125 mL serum bottle;
7. Crimp all serum bottles;
8. Flush all serum bottles with nitrogen;
9. Incubate all 125 mL serum bottles in the shaking incubator at 250 rpm and 33^{o}C for 24 hours;
10. Take samples to be analyzed for sugars and ethanol concentration by HPLC at 0, 6, 12, 24 and 48 hour;
11. Calculate the % theoretical ethanol yield by using the following formula:

$$\% \text{ theoretical ethanol yield} = \frac{[EtOH]f - [EtOH]i}{0.51(f[Biomass]1.111)} \times 100$$

where $[EtOH]_f$ - ethanol conc. at the end of fermentation, (g/L); [EtOH]i - ethanol conc. at the beginning of fermentation, (g/L); [Biomass] - dry biomass conc. at the beginning of fermentation, (g/L); f - Cellulose fraction of dry biomass, (g/g); 0.51 - Conversion factor for glucose to ethanol; 1.111 –conversion factor for cellulose to equivalent glucose

Procedure for HPLC Sample Preparation:

1. Mobile phase must be prepared fresh for each run. The first two HPLC vials are buffer containing only mobile phase contents.
2. Prepare duplicate standards for each run.
3. Centrifuge sample in 2 mL centrifuge tubes at 10,000 rcf for 5 minutes to remove protein and particulates, then filter (0.22 um) about 1 mL of the supernatant into HPLC vial. Sample can be stored in the fridge for a few days if not used immediately.
4. Instrumental conditions for HPX-87P column:
    - Sample volume: 50uL.
    - Eluant: 0.2 um filtered and degassed, deionized water.
    - Flow rate: 0.6 mL/min.
    - Column temperature: 85^{o}C.
    - Detector: refractive index.
    - Run time: 15 minutes data collection plus a 75 minute pump ramp.
5. Instrumental conditions for HPX-87H column:
    - Sample volume: 50uL.
    - Eluant: 0.2 um filtered and degassed, 0.005 M sulfuric acid.
    - Flow rate: 0.6 mL/min.
    - Column temperature: 65^{o}C.
    - Detector: refractive index.
    - Run time: 25 minutes data collection plus a 75 minute pump ramp.

APPENDIX E: HPLC SAMPLES RUNS FOR ENZYMATIC HYDROLYSIS AND FERMENTATION

Software Version	: 6.3.2.0646	Date	: 6/10/2013 6:26:57 PM
Operator	: rLuong	Sample Name	: S1
Sample Number	: 008	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/8
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 3:28:05 PM	Dilution Factor	: 1.00
		Cycle	: 8

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7008.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7008.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7008.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7008.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7008.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.527	765494.40	4282.17	6.70	6.70	BB	178.7633
2	10.007	115320.80	4061.00	1.01	1.01	BB	28.3971
3	10.947	18559.20	1325.00	0.16	0.16	BB	14.0069
4	11.707	90122.44	5542.38	0.79	0.79	BB	16.2606
5	12.607	10392161.96	373747.35	90.95	90.95	MM	27.8053
6	13.700	44735.00	2414.89	0.39	0.39	BB	18.5246
		11426393.80	391372.79	100.00	100.00		

Warning -- Signal level out-of-range in peak

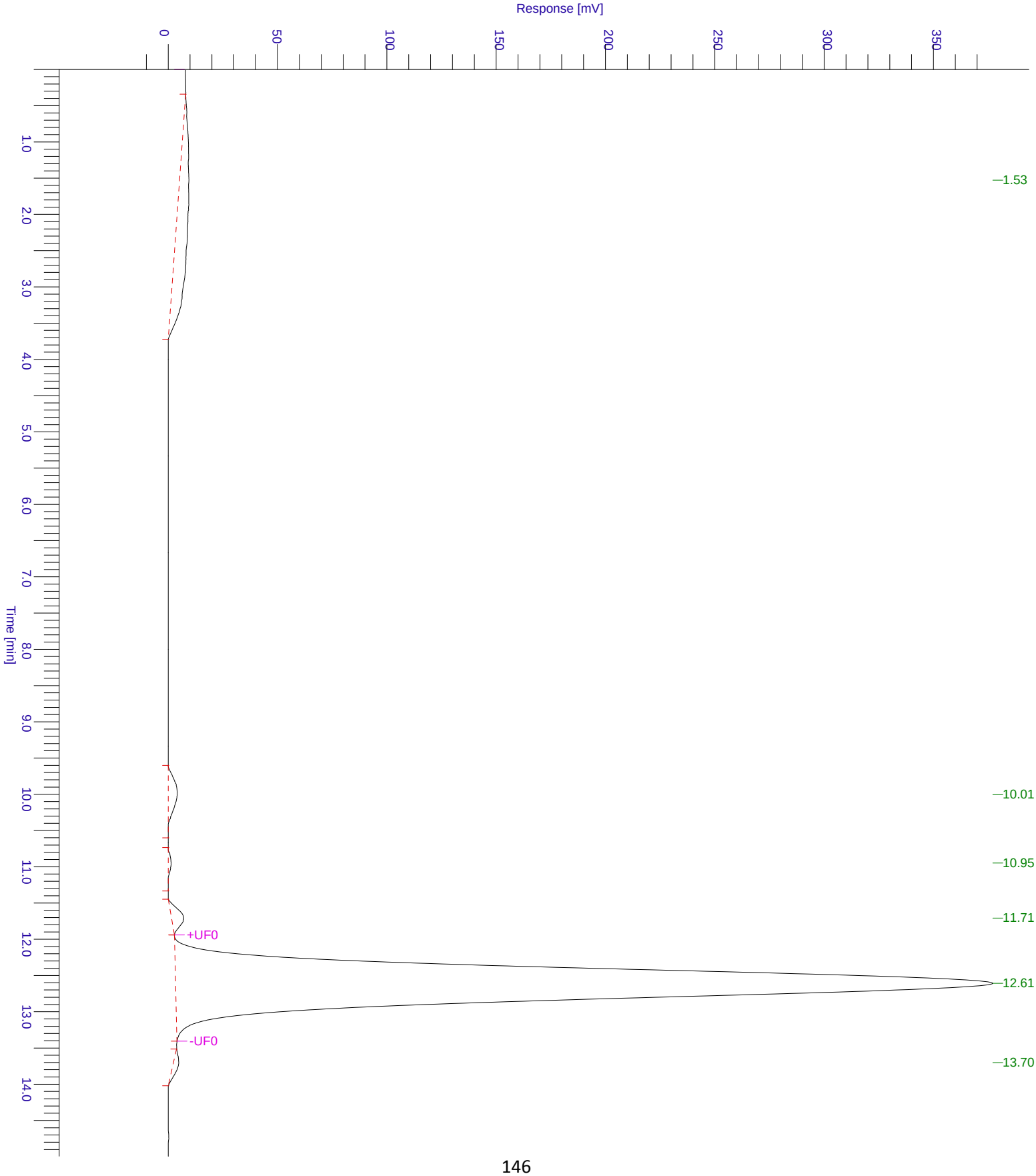
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S1 Sample #: 008 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7008.raw
Date : 6/10/2013 6:27:52 PM
Method : Method Robin 87P Time of Injection: 6/7/2013 3:28:05 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -18.86 mV High Point : 377.12 mV
Scale Factor: 1.0 Plot Offset: -18.86 mV Plot Scale: 396.0 mV



Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:16:20 AM
Operator	: rLuong	Sample Name	: S10
Sample Number	: 017	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/17
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 5:52:13 PM	Dilution Factor	: 1.00
		Cycle	: 17

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7017.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7017.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7017.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7017.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7017.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.958	13808760.60	560482.86	60.13	60.13	BB	24.6373
2	4.770	79620.60	912.36	0.35	0.35	BB	87.2688
3	6.558	64882.33	2453.45	0.28	0.28	BV	26.4453
4	7.093	165407.24	5974.69	0.72	0.72	VV	27.6847
5	9.976	543415.43	7866.00	2.37	2.37	VB	69.0841
6	11.673	127840.80	6725.96	0.56	0.56	BB	19.0071
7	12.600	8111969.32	290151.09	35.32	35.32	MM	27.9577
8	13.686	41986.00	1979.95	0.18	0.18	BB	21.2056
9	14.729	20678.80	1035.69	0.09	0.09	BB	19.9661
		22964561.12	877582.06	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S10 Sample #: 017 Page 1 of 1
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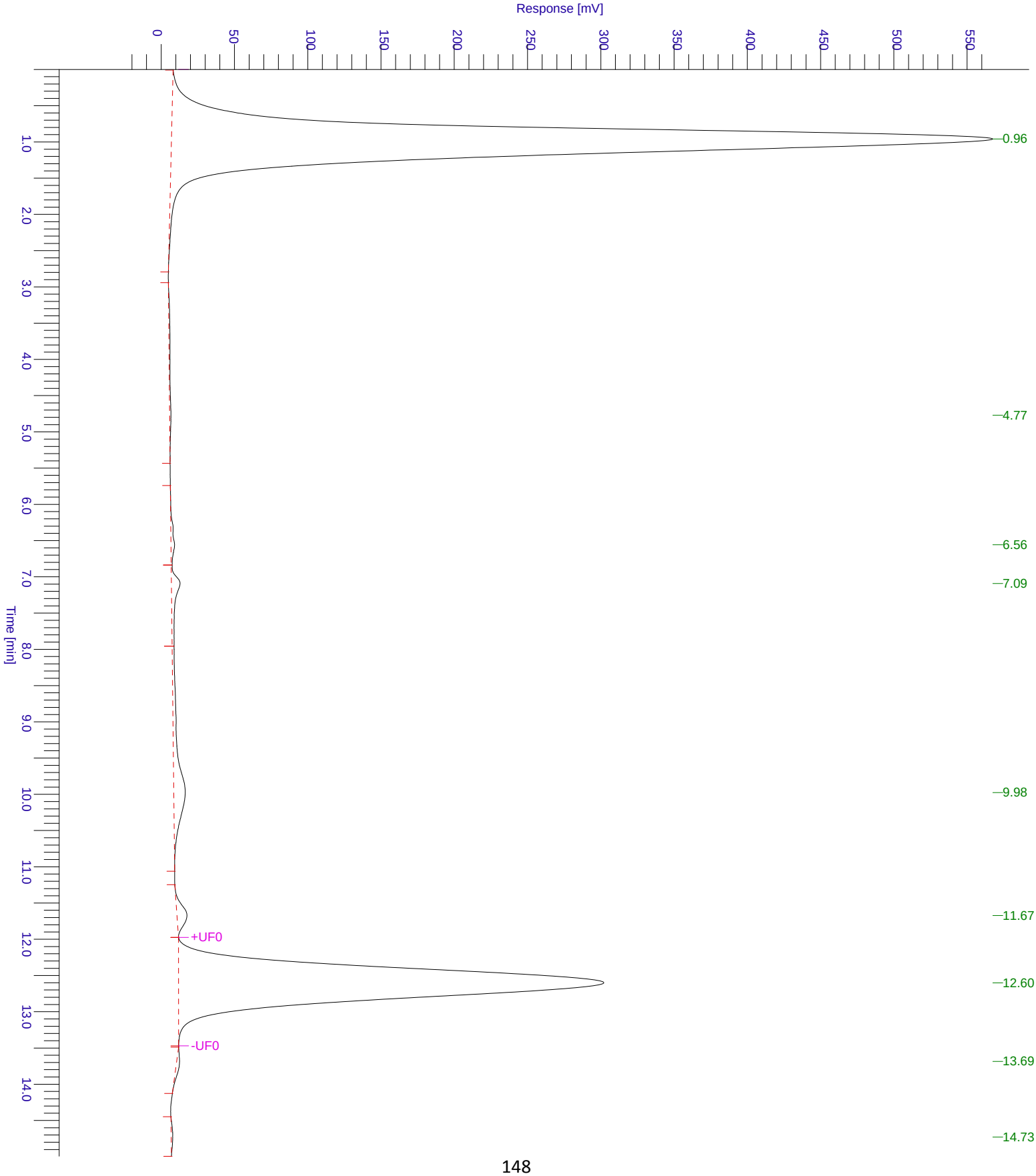
Date : 6/11/2013 11:16:46 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 5:52:13 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -23.33 mV High Point : 567.48 mV
Scale Factor: 1.0 Plot Offset: -23.33 mV Plot Scale: 590.8 mV



Software Version	: 6.3.2.0646	Date	: 6/10/2013 6:28:50 PM
Operator	: rLuong	Sample Name	: S2
Sample Number	: 009	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/9
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 3:44:06 PM	Dilution Factor	: 1.00
		Cycle	: 9

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7009.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7009.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7009.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7009.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7009.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.961	13768516.80	557905.49	55.59	55.59	BB	24.6789
2	6.583	66255.20	2265.80	0.27	0.27	BB	29.2414
3	7.093	118781.76	5859.92	0.48	0.48	BV	20.2702
4	9.984	363543.40	7016.34	1.47	1.47	VV	51.8138
5	10.962	76140.62	2944.20	0.31	0.31	VB	25.8613
6	11.711	103361.60	5982.34	0.42	0.42	BB	17.2778
7	12.613	10182019.12	366144.60	41.11	41.11	MM	27.8087
8	13.689	51729.20	2498.78	0.21	0.21	BB	20.7018
9	14.721	39064.80	1858.74	0.16	0.16	BB	21.0168
		24769412.51	952476.21	100.00	100.00		

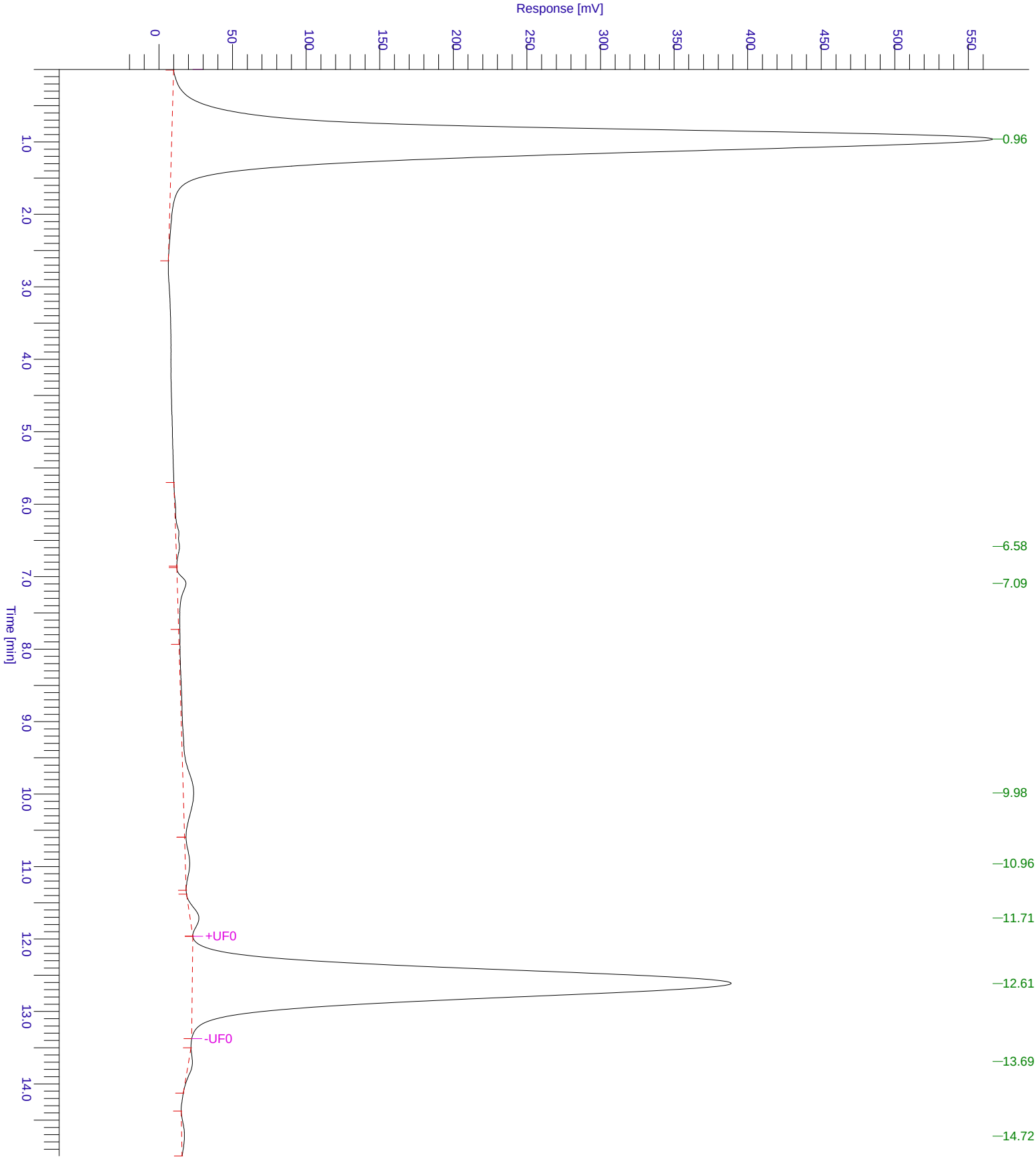
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S2 Sample #: 009 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7009.raw
Date : 6/10/2013 6:29:34 PM
Method : Method Robin 87P Time of Injection: 6/7/2013 3:44:06 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -21.72 mV High Point : 566.53 mV
Scale Factor: 1.0 Plot Offset: -21.72 mV Plot Scale: 588.3 mV



Software Version	: 6.3.2.0646	Date	: 6/10/2013 6:31:01 PM
Operator	: rLuong	Sample Name	: S3
Sample Number	: 010	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/10
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 4:00:10 PM	Dilution Factor	: 1.00
		Cycle	: 10

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7010.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7010.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7010.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7010.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7010.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.902	13966610.40	568541.53	58.46	58.46	BB	24.5657
2	3.623	68713.60	1110.13	0.29	0.29	BB	61.8969
3	6.374	39718.40	1681.31	0.17	0.17	BB	23.6235
4	7.093	98591.20	5313.00	0.41	0.41	BB	18.5566
5	9.968	292326.64	6327.39	1.22	1.22	BV	46.2002
6	10.982	27547.56	1284.56	0.12	0.12	VB	21.4452
7	11.710	108306.80	6252.86	0.45	0.45	BB	17.3212
8	12.613	9189055.08	331215.49	38.46	38.46	MM	27.7434
9	13.692	62056.40	2741.10	0.26	0.26	BB	22.6392
10	14.715	37177.20	1858.03	0.16	0.16	BB	20.0089
		23890103.28	926325.40	100.00	100.00		

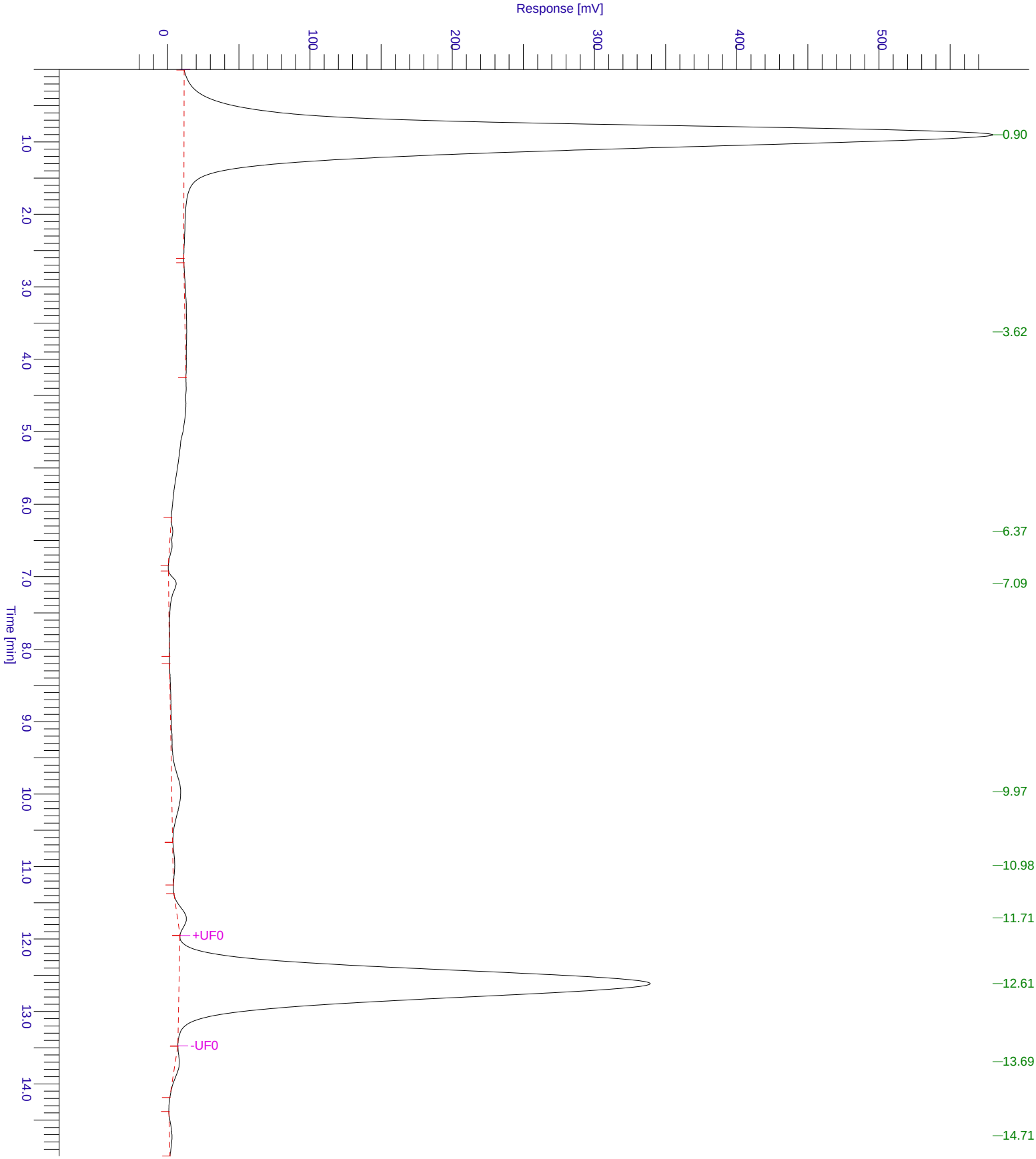
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S3 Sample #: 010 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7010.raw
Date : 6/10/2013 6:31:32 PM
Method : Method Robin 87P Time of Injection: 6/7/2013 4:00:10 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -28.60 mV High Point : 579.94 mV
Scale Factor: 1.0 Plot Offset: -28.60 mV Plot Scale: 608.5 mV



Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:08:38 AM
Operator	: rLuong	Sample Name	: S4
Sample Number	: 011	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/11
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 4:16:12 PM	Dilution Factor	: 1.00
		Cycle	: 11

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7011.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7011.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7011.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7011.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7011.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.930	13931651.40	564347.27	61.27	61.27	BB	24.6863
2	3.736	53043.18	783.74	0.23	0.23	BV	67.6799
3	4.711	27901.22	774.65	0.12	0.12	VB	36.0177
4	6.566	77047.60	2669.79	0.34	0.34	BV	28.8590
5	7.090	160632.13	6304.13	0.71	0.71	VV	25.4805
6	9.971	496745.28	7470.19	2.18	2.18	VB	66.4970
7	11.711	124848.40	6825.90	0.55	0.55	BB	18.2904
8	12.607	7765087.75	279818.61	34.15	34.15	MM	27.7504
9	13.678	62746.40	2681.57	0.28	0.28	BB	23.3992
10	14.742	38408.00	1824.29	0.17	0.17	BB	21.0536
		22738111.35	873500.15	100.00	100.00		

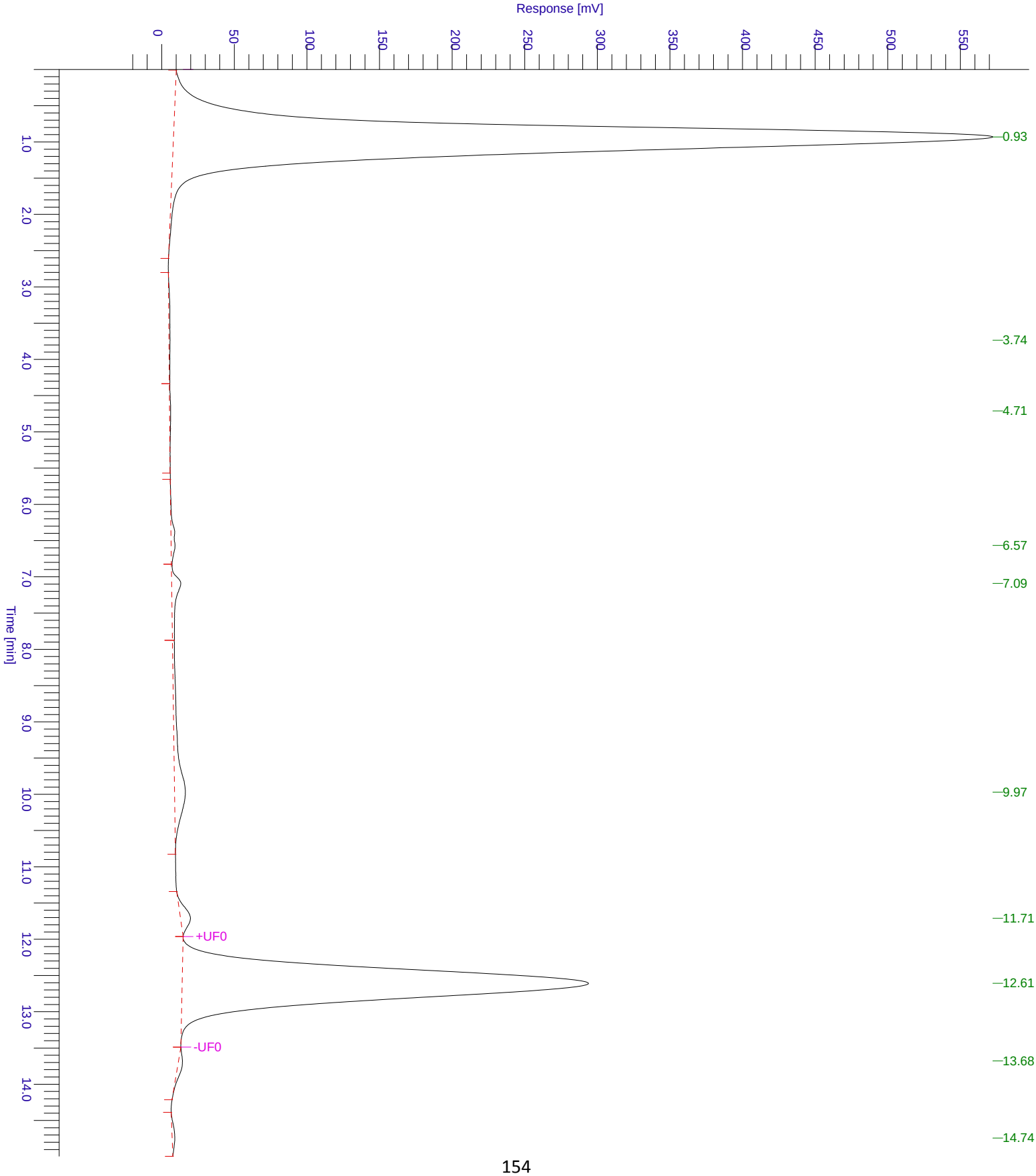
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S4 Sample #: 011 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7011.raw
Date : 6/11/2013 11:09:21 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 4:16:12 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -23.88 mV High Point : 572.31 mV
Scale Factor: 1.0 Plot Offset: -23.88 mV Plot Scale: 596.2 mV



Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:10:19 AM
Operator	: rLuong	Sample Name	: S5
Sample Number	: 012	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/12
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 4:32:12 PM	Dilution Factor	: 1.00
		Cycle	: 12

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7012.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7012.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7012.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7012.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7012.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.964	14439082.00	583336.43	73.39	73.39	BB	24.7526
2	3.773	125110.40	3431.42	0.64	0.64	BB	36.4603
3	6.578	71493.32	2539.12	0.36	0.36	BV	28.1567
4	7.101	135619.99	5888.98	0.69	0.69	VV	23.0294
5	9.992	449335.33	6719.11	2.28	2.28	VB	66.8742
6	11.714	120743.60	6384.70	0.61	0.61	BB	18.9114
7	12.620	4223898.23	152384.21	21.47	21.47	MM	27.7187
8	13.715	73686.20	3259.28	0.37	0.37	BB	22.6081
9	14.760	35343.60	1661.33	0.18	0.18	BB	21.2743
		19674312.67	765604.57	100.00	100.00		

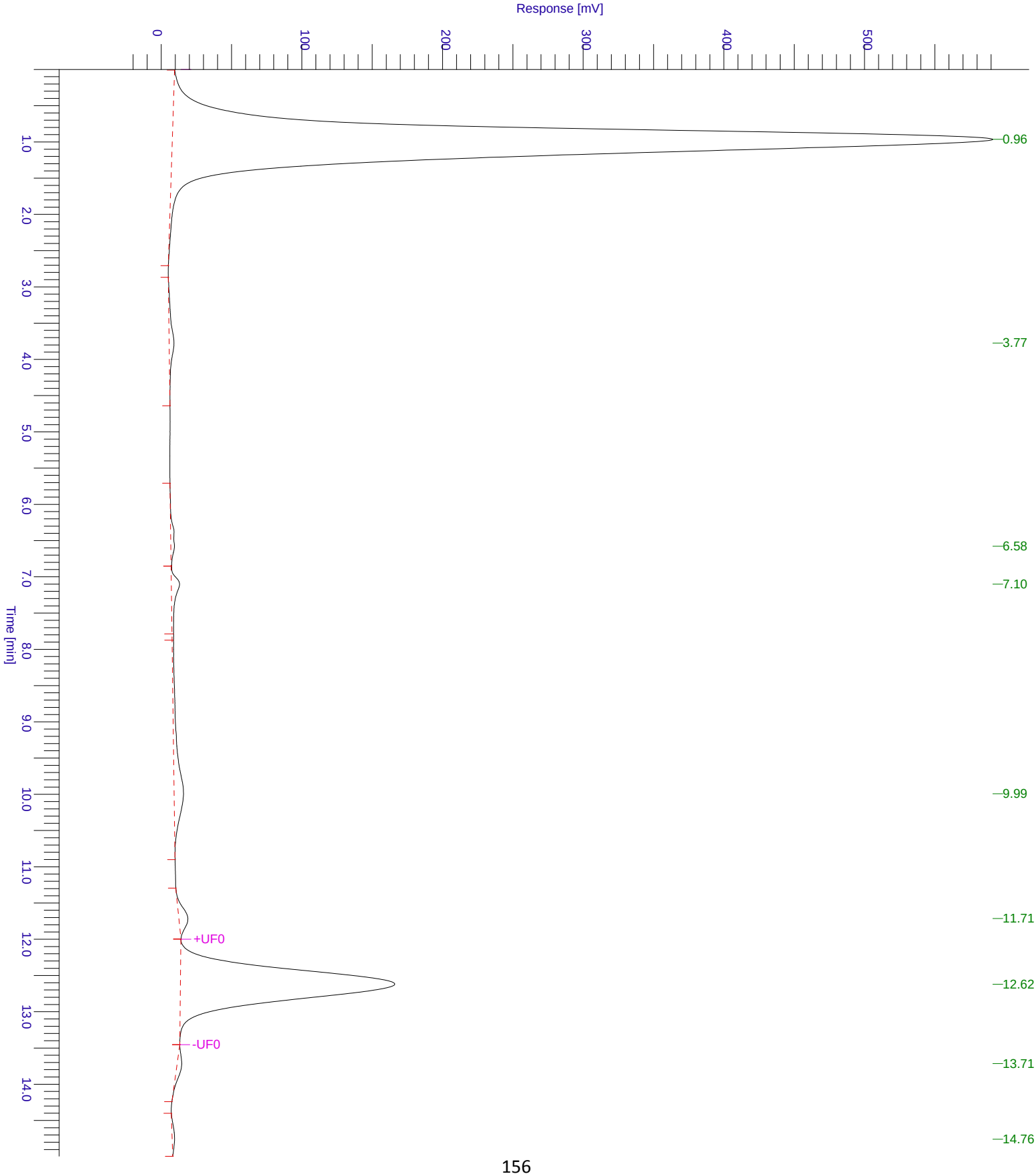
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S5 Sample #: 012 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7012.raw
Date : 6/11/2013 11:10:45 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 4:32:12 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -24.33 mV High Point : 591.17 mV
Scale Factor: 1.0 Plot Offset: -24.33 mV Plot Scale: 615.5 mV



Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:11:39 AM
Operator	: rLuong	Sample Name	: S6
Sample Number	: 013	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/13
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 4:48:13 PM	Dilution Factor	: 1.00
		Cycle	: 13

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7013.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7013.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7013.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7013.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7013.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.965	14634117.20	591146.50	84.99	84.99	BB	24.7555
2	3.783	350603.60	11652.28	2.04	2.04	BB	30.0888
3	6.564	75987.44	2654.65	0.44	0.44	BV	28.6242
4	7.103	141886.73	5800.29	0.82	0.82	VV	24.4620
5	9.999	436990.48	6541.62	2.54	2.54	VB	66.8016
6	11.719	81642.80	4185.48	0.47	0.47	BB	19.5062
7	12.613	1357805.17	49899.34	7.89	7.89	MM	27.2109
8	13.713	100710.80	3828.30	0.58	0.58	BB	26.3069
9	14.754	38837.20	1873.16	0.23	0.23	BB	20.7335
		17218581.42	677581.62	100.00	100.00		

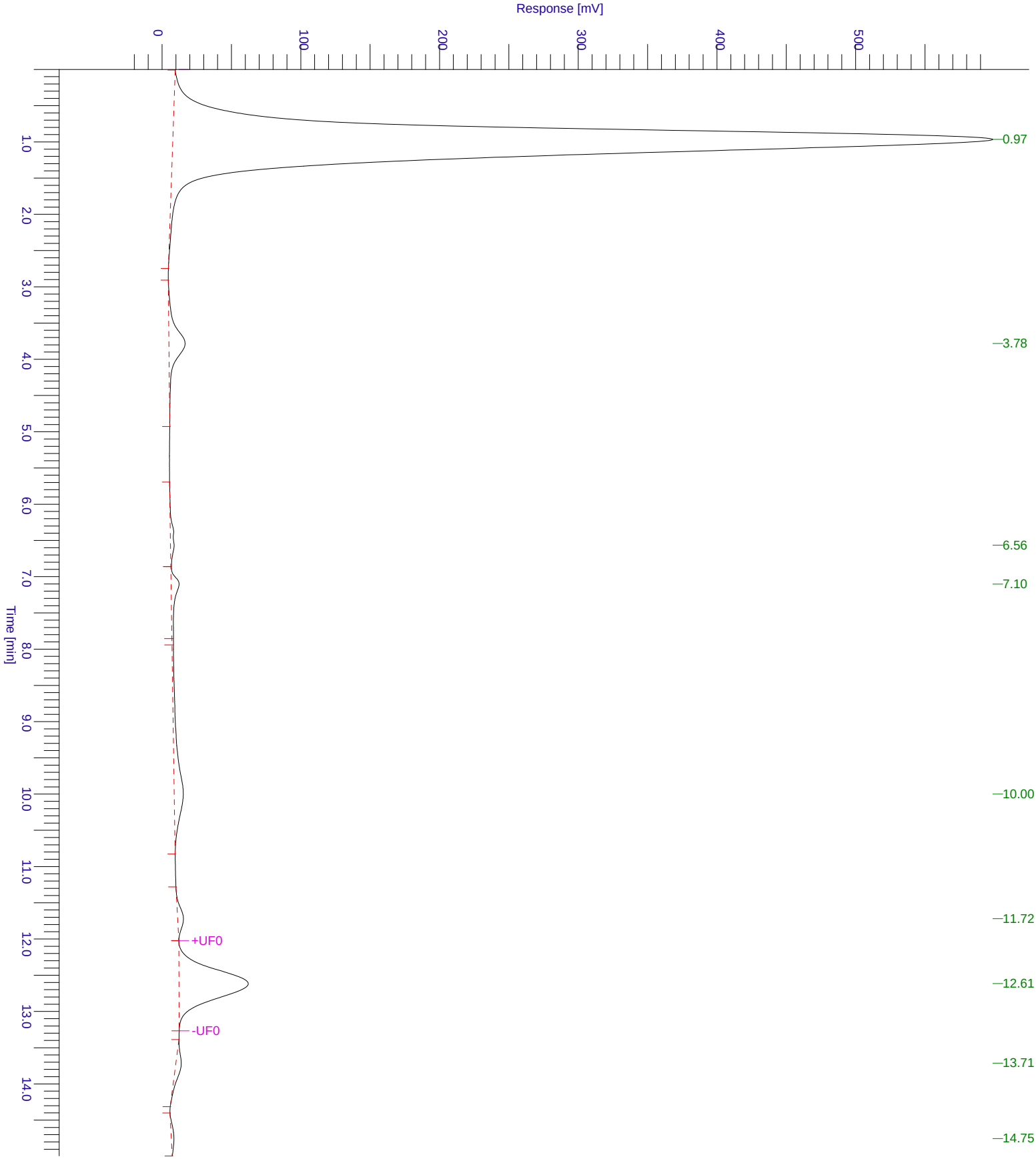
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S6 Sample #: 013 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7013.raw
Date : 6/11/2013 11:12:01 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 4:48:13 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -25.29 mV High Point : 598.82 mV
Scale Factor: 1.0 Plot Offset: -25.29 mV Plot Scale: 624.1 mV



Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:13:20 AM
Operator	: rLuong	Sample Name	: S7
Sample Number	: 014	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/14
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 5:04:13 PM	Dilution Factor	: 1.00
		Cycle	: 14

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7014.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7014.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7014.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7014.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7014.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.968	14884357.40	601287.55	57.54	57.54	BB	24.7541
2	3.789	529471.60	16556.23	2.05	2.05	BB	31.9802
3	6.580	61092.18	2194.09	0.24	0.24	BV	27.8440
4	7.091	150174.56	5379.82	0.58	0.58	VV	27.9144
5	9.960	509931.00	7471.14	1.97	1.97	VV	68.2535
6	10.925	46912.02	2031.27	0.18	0.18	VB	23.0950
7	11.709	90303.00	5184.21	0.35	0.35	BB	17.4189
8	12.607	9593459.50	342631.55	37.09	37.09	MM	27.9993
		25865701.27	982735.86	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S7 Sample #: 014 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7014.raw
Date : 6/11/2013 11:13:43 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 5:04:13 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -27.14 mV High Point : 608.90 mV
Scale Factor: 1.0 Plot Offset: -27.14 mV Plot Scale: 636.0 mV

Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:14:24 AM
Operator	: rLuong	Sample Name	: S8
Sample Number	: 015	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/15
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 5:20:13 PM	Dilution Factor	: 1.00
		Cycle	: 15

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7015.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7015.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7015.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7015.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7015.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.964	12217116.00	500394.26	53.52	53.52	BB	24.4150
2	3.756	23028.00	454.29	0.10	0.10	BB	50.6903
3	6.559	51681.99	2042.10	0.23	0.23	BV	25.3083
4	7.105	125563.14	5324.94	0.55	0.55	VV	23.5802
5	9.983	419991.56	6876.46	1.84	1.84	VV	61.0767
6	10.927	58621.32	2161.37	0.26	0.26	VB	27.1224
7	11.706	79564.80	5023.85	0.35	0.35	BB	15.8374
8	12.607	9853687.31	349773.56	43.16	43.16	MM	28.1716
		22829254.11	872050.83	100.00	100.00		

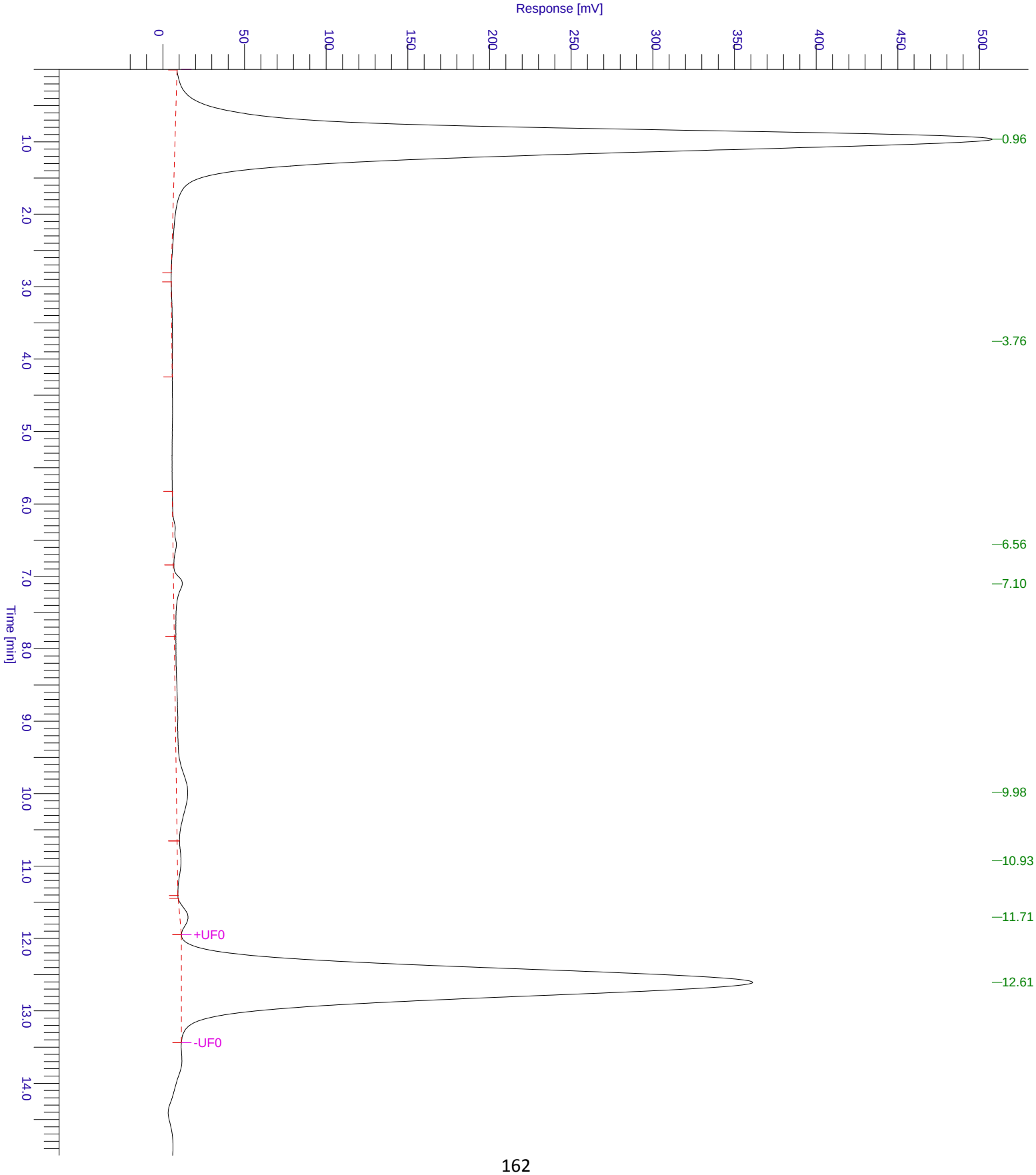
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S8 Sample #: 015 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7015.raw
Date : 6/11/2013 11:14:45 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 5:20:13 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -21.99 mV High Point : 507.73 mV
Scale Factor: 1.0 Plot Offset: -21.99 mV Plot Scale: 529.7 mV



Software Version	: 6.3.2.0646	Date	: 6/11/2013 11:15:23 AM
Operator	: rLuong	Sample Name	: S9
Sample Number	: 016	Study	: Glucose
AutoSampler	: SER200	Rack/Vial	: 1/16
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 14.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/7/2013 5:36:13 PM	Dilution Factor	: 1.00
		Cycle	: 16

Raw Data File : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7016.raw
Result File : c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7016.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87P from C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7016.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7016.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87P from c:\hplc data\robin\june 7-13_glucose\eh_glucose_june 7016.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87p-glucose-June 7-13.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.960	13189985.80	536040.70	55.74	55.74	BB	24.6063
2	4.798	81742.00	976.84	0.35	0.35	BB	83.6802
3	6.565	51286.97	2143.79	0.22	0.22	BV	23.9235
4	7.093	156863.30	5625.16	0.66	0.66	VV	27.8860
5	9.981	476257.73	7375.34	2.01	2.01	VB	64.5744
6	11.701	103874.40	5644.53	0.44	0.44	BB	18.4027
7	12.607	9579356.30	340995.65	40.48	40.48	MM	28.0923
8	14.750	26009.09	1313.68	0.11	0.11	BB	19.7987
		23665375.58	900115.67	100.00	100.00		

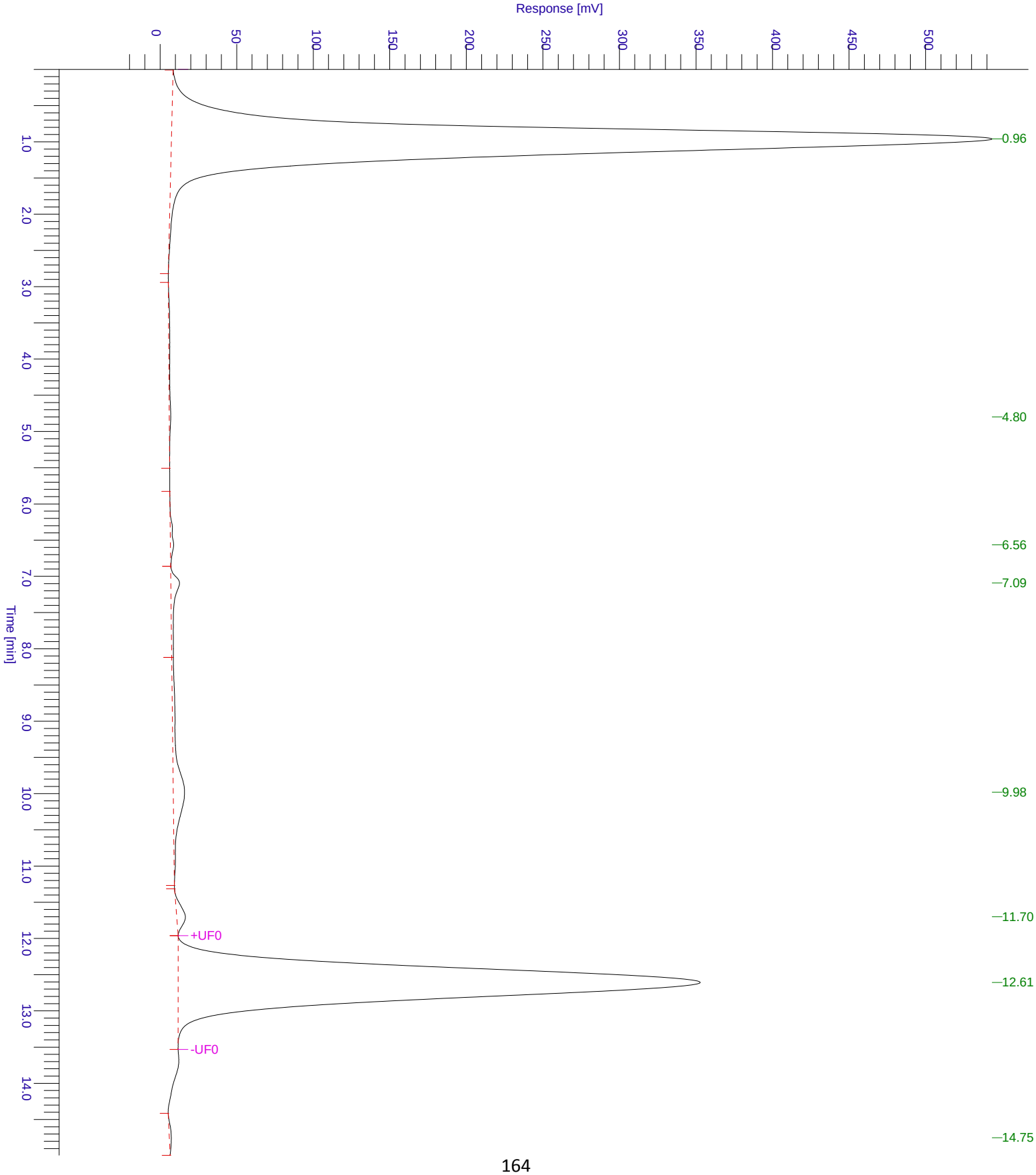
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : S9 Sample #: 016 Page 1 of 1
FileName : C:\HPLC Data\robin\June 7-13_Glucose\EH_Glucose_June 7016.raw
Date : 6/11/2013 11:15:46 AM
Method : Method Robin 87P Time of Injection: 6/7/2013 5:36:13 PM
Start Time : 0.00 min End Time : 14.99 min Low Point : -21.69 mV High Point : 543.42 mV
Scale Factor: 1.0 Plot Offset: -21.69 mV Plot Scale: 565.1 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:48:01 PM
Operator	: rLuong	Sample Name	: S1
Sample Number	: 008	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/8
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 4:23:49 PM	Dilution Factor	: 1.00
		Cycle	: 8

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol008.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol008.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol008.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol008.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol008.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.227	354.80	45.51	0.00	0.00	BB	7.7957
2	0.340	275.00	59.88	0.00	0.00	BB	4.5923
3	0.802	146.00	66.24	0.00	0.00	BB	2.2042
4	0.976	76.20	51.69	7e-05	7e-05	BB	1.4740
5	1.174	306.80	56.56	0.00	0.00	BB	5.4243
6	1.452	313.20	65.50	0.00	0.00	BB	4.7818
7	2.098	145.00	61.95	0.00	0.00	BB	2.3407
8	2.405	183.40	61.17	0.00	0.00	BB	2.9984
9	2.506	151.60	89.16	0.00	0.00	BB	1.7003
10	2.572	207.20	103.39	0.00	0.00	BB	2.0042
11	2.640	101.20	41.18	9e-05	9e-05	BB	2.4576
12	2.786	257.20	67.01	0.00	0.00	BB	3.8381
13	2.849	200.35	112.91	0.00	0.00	BV	1.7744
14	2.906	306.05	86.85	0.00	0.00	VB	3.5239
15	2.990	98.20	75.11	9e-05	9e-05	BB	1.3074
16	3.221	70.20	63.19	6e-05	6e-05	BB	1.1109
17	3.705	196.00	94.34	0.00	0.00	BB	2.0777
18	3.855	172.40	82.58	0.00	0.00	BB	2.0877
19	3.986	613.82	90.56	0.00	0.00	BV	6.7781
20	4.118	207.63	88.30	0.00	0.00	VV	2.3514
21	4.192	301.02	83.40	0.00	0.00	VV	3.6095
22	4.248	147.33	80.52	0.00	0.00	VB	1.8297
23	4.300	76.00	66.24	7e-05	7e-05	BB	1.1473
24	4.385	97.60	73.69	9e-05	9e-05	BB	1.3244
25	4.658	475.60	113.03	0.00	0.00	BB	4.2078
26	4.764	50.45	68.72	5e-05	5e-05	BV	0.7341
27	4.792	453.55	80.92	0.00	0.00	VB	5.6051
28	5.062	393.20	67.29	0.00	0.00	BB	5.8433
29	5.321	455.60	89.48	0.00	0.00	BB	5.0915
30	5.417	47.60	66.73	4e-05	4e-05	BB	0.7133
31	6.277	53455.53	8595.70	0.05	0.05	BV	6.2189
32	6.583	5482159.97	223020.29	5.02	5.02	VV	24.5814
33	7.293	3382643.22	92756.48	3.10	3.10	VV	36.4680
34	8.183	903827.98	59424.96	0.83	0.83	VV	15.2096
35	8.601	13702074.69	580668.14	12.55	12.55	VV	23.5971
36	9.267	54703933.54	991695.74	50.10	50.10	VE	55.1620
37	11.730	743485.20	12509.48	0.68	0.68	EV	59.4338
38	13.358	137453.68	4187.75	0.13	0.13	VB	32.8228
39	14.778	77.20	46.23	7e-05	7e-05	BB	1.6700
40	15.336	16930.87	1272.04	0.02	0.02	BV	13.3100
41	15.385	14869.33	1240.23	0.01	0.01	VB	11.9892
42	15.965	250.40	96.88	0.00	0.00	BB	2.5846
43	16.388	323.60	101.36	0.00	0.00	BB	3.1927
44	16.955	68.40	56.59	6e-05	6e-05	BB	1.2086
45	17.424	93.80	37.11	9e-05	9e-05	BB	2.5277

6/21/2013 12:48:01 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol008.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	17.523	100.80	49.41	9e-05	9e-05	BB	2.0402
47	17.834	85.00	36.86	8e-05	8e-05	BB	2.3063
48	17.918	201.09	83.50	0.00	0.00	BV	2.4081
49	18.309	6651.11	417.28	0.01	0.01	VB	15.9391
50	18.888	142.00	55.11	0.00	0.00	BB	2.5765
51	19.300	584.40	45.27	0.00	0.00	BB	12.9099
52	19.439	231.40	46.68	0.00	0.00	BB	4.9576
53	20.077	155.42	59.99	0.00	0.00	BV	2.5909
54	20.524	2688.23	180.16	0.00	0.00	VV	14.9209
55	20.578	681.48	198.33	0.00	0.00	VV	3.4361
56	21.539	30032187.67	753475.41	27.50	27.50	VE	39.8582
57	23.291	773.20	82.90	0.00	0.00	EB	9.3275
58	23.615	633.20	70.43	0.00	0.00	BB	8.9899
59	23.771	372.60	90.04	0.00	0.00	BB	4.1381
60	24.037	302.40	79.50	0.00	0.00	BB	3.8037
61	24.210	33.60	34.85	3e-05	3e-05	BB	0.9640
62	24.611	56.80	48.53	5e-05	5e-05	BB	1.1703
63	24.889	164.80	74.44	0.00	0.00	BB	2.2138
		1.09e+08	2.73e+06	100.00	100.00		

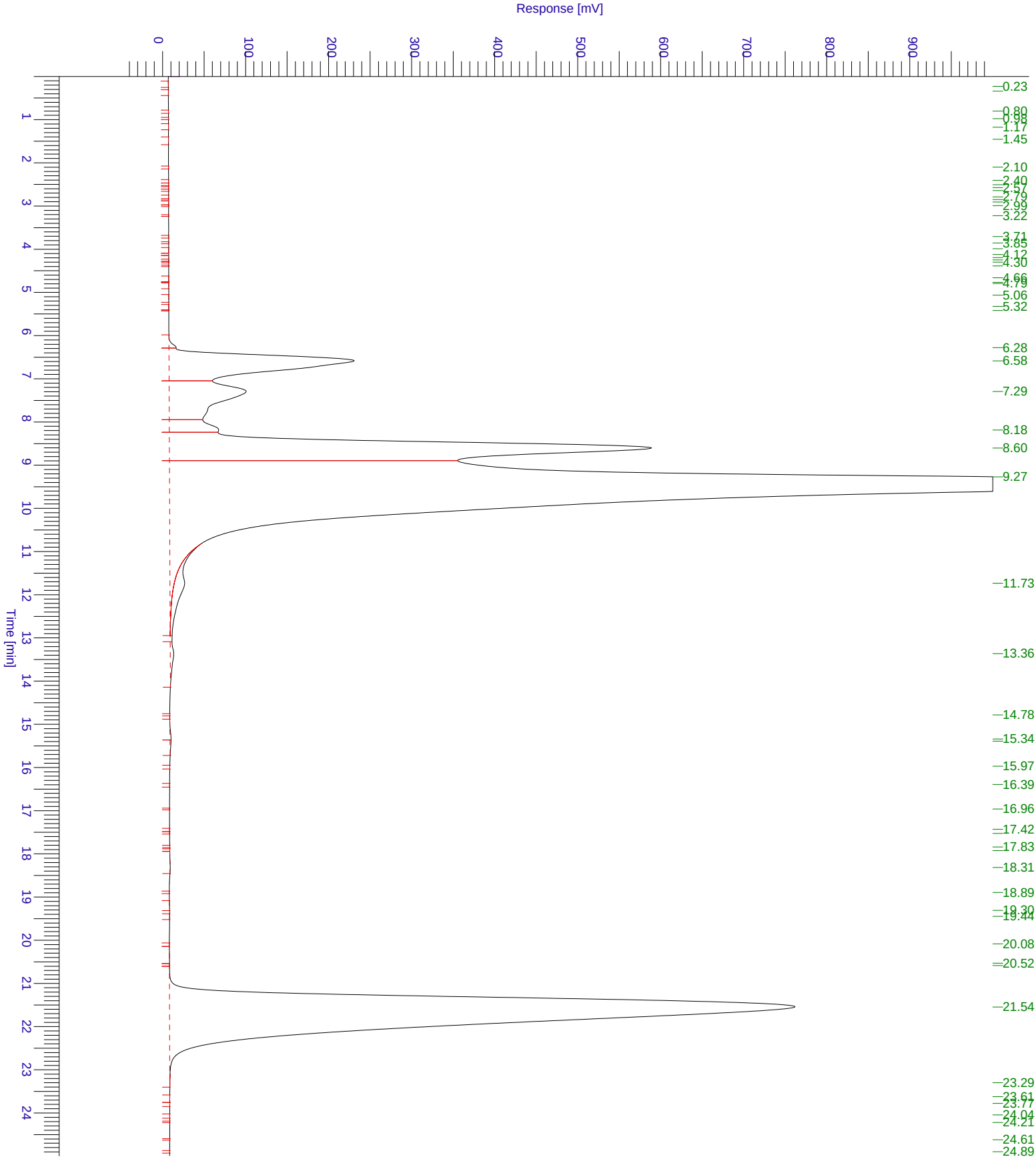
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S1 Sample #: 008 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol008.raw
Date : 6/21/2013 12:48:31 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 4:23:49 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -42.86 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -42.86 mV Plot Scale: 1042.9 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:57:56 PM
Operator	: rLuong	Sample Name	: S10
Sample Number	: 017	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/17
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 8:20:13 PM	Dilution Factor	: 1.00
		Cycle	: 17

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol017.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol017.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol017.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol017.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol017.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.025	84.40	50.71	8e-05	8e-05	BB	1.6645
2	0.255	1339.60	143.84	0.00	0.00	BB	9.3129
3	0.504	165.20	92.60	0.00	0.00	BB	1.7840
4	0.731	205.20	38.58	0.00	0.00	BB	5.3184
5	2.177	143.20	36.47	0.00	0.00	BB	3.9265
6	2.499	135.80	32.16	0.00	0.00	BB	4.2229
7	2.820	161.20	37.32	0.00	0.00	BB	4.3200
8	3.145	207.27	38.91	0.00	0.00	BV	5.3271
9	3.301	156.33	37.33	0.00	0.00	VB	4.1879
10	3.463	72.00	26.47	7e-05	7e-05	BB	2.7201
11	3.784	137.60	31.13	0.00	0.00	BB	4.4197
12	4.106	148.00	33.66	0.00	0.00	BB	4.3975
13	4.583	61.20	25.23	6e-05	6e-05	BB	2.4253
14	6.568	3099266.35	109465.00	3.03	3.03	BV	28.3129
15	7.303	3307798.33	90922.22	3.24	3.24	VV	36.3805
16	8.610	17241122.16	691790.70	16.87	16.87	VV	24.9225
17	9.320	49904628.16	997531.66	48.83	48.83	VB	50.0281
18	13.413	174493.60	14190.00	0.17	0.17	BB	12.2969
19	13.718	675894.40	33875.01	0.66	0.66	BB	19.9526
20	14.862	189.63	97.37	0.00	0.00	BV	1.9475
21	15.369	634771.97	19884.35	0.62	0.62	VB	31.9232
22	16.615	244.40	50.88	0.00	0.00	BB	4.8039
23	16.779	157.20	84.93	0.00	0.00	BB	1.8510
24	16.910	66.00	41.40	6e-05	6e-05	BB	1.5943
25	17.010	179.20	81.71	0.00	0.00	BB	2.1931
26	17.256	356.80	130.37	0.00	0.00	BB	2.7368
27	17.510	199.40	78.66	0.00	0.00	BB	2.5349
28	17.577	163.20	76.15	0.00	0.00	BB	2.1431
29	17.802	563.67	129.40	0.00	0.00	BV	4.3560
30	17.922	1918.37	220.97	0.00	0.00	VV	8.6816
31	18.029	512.19	281.15	0.00	0.00	VV	1.8218
32	18.094	2847.28	399.97	0.00	0.00	VV	7.1188
33	18.212	3075.29	362.83	0.00	0.00	VB	8.4758
34	18.763	79.20	62.23	8e-05	8e-05	BB	1.2728
35	19.286	98856.52	2921.24	0.10	0.10	BE	33.8406
36	19.919	788.80	104.83	0.00	0.00	EV	7.5248
37	20.030	322.28	149.18	0.00	0.00	VB	2.1603
38	20.084	127.60	92.00	0.00	0.00	BB	1.3870
39	20.196	131.20	104.86	0.00	0.00	BB	1.2512
40	20.234	372.40	103.06	0.00	0.00	BB	3.6133
41	20.314	131.00	134.02	0.00	0.00	BB	0.9775
42	20.483	258.80	100.34	0.00	0.00	BB	2.5793
43	20.531	298.40	152.42	0.00	0.00	BB	1.9577
44	20.602	94.60	98.32	9e-05	9e-05	BB	0.9622
45	20.637	409.67	131.79	0.00	0.00	BV	3.1085

6/21/2013 12:57:56 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol017.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	20.762	808.98	266.36	0.00	0.00	VV	3.0372
47	20.806	613.24	337.95	0.00	0.00	VV	1.8146
48	21.538	27048584.91	682579.76	26.46	26.46	VE	39.6270
49	22.877	668.40	142.44	0.00	0.00	EB	4.6924
50	23.199	348.40	99.94	0.00	0.00	BB	3.4860
51	23.311	47.20	66.32	5e-05	5e-05	BB	0.7117
52	23.364	141.20	59.72	0.00	0.00	BB	2.3644
53	23.502	453.54	144.48	0.00	0.00	BV	3.1390
54	23.575	188.57	129.51	0.00	0.00	VV	1.4560
55	23.618	117.89	98.91	0.00	0.00	VB	1.1919
56	23.665	58.00	79.18	6e-05	6e-05	BB	0.7325
57	24.031	416.00	160.43	0.00	0.00	BB	2.5930
58	24.146	36.40	49.41	4e-05	4e-05	BB	0.7367
59	24.221	203.20	102.58	0.00	0.00	BB	1.9809
60	24.424	1149.00	179.38	0.00	0.00	BV	6.4055
61	24.558	397.40	129.82	0.00	0.00	VB	3.0613
62	24.647	205.60	103.30	0.00	0.00	BB	1.9903
63	24.830	504.80	68.88	0.00	0.00	BB	7.3284
64	24.974	108.40	58.59	0.00	0.00	BB	1.8502
		1.02e+08	2.65e+06	100.00	100.00		

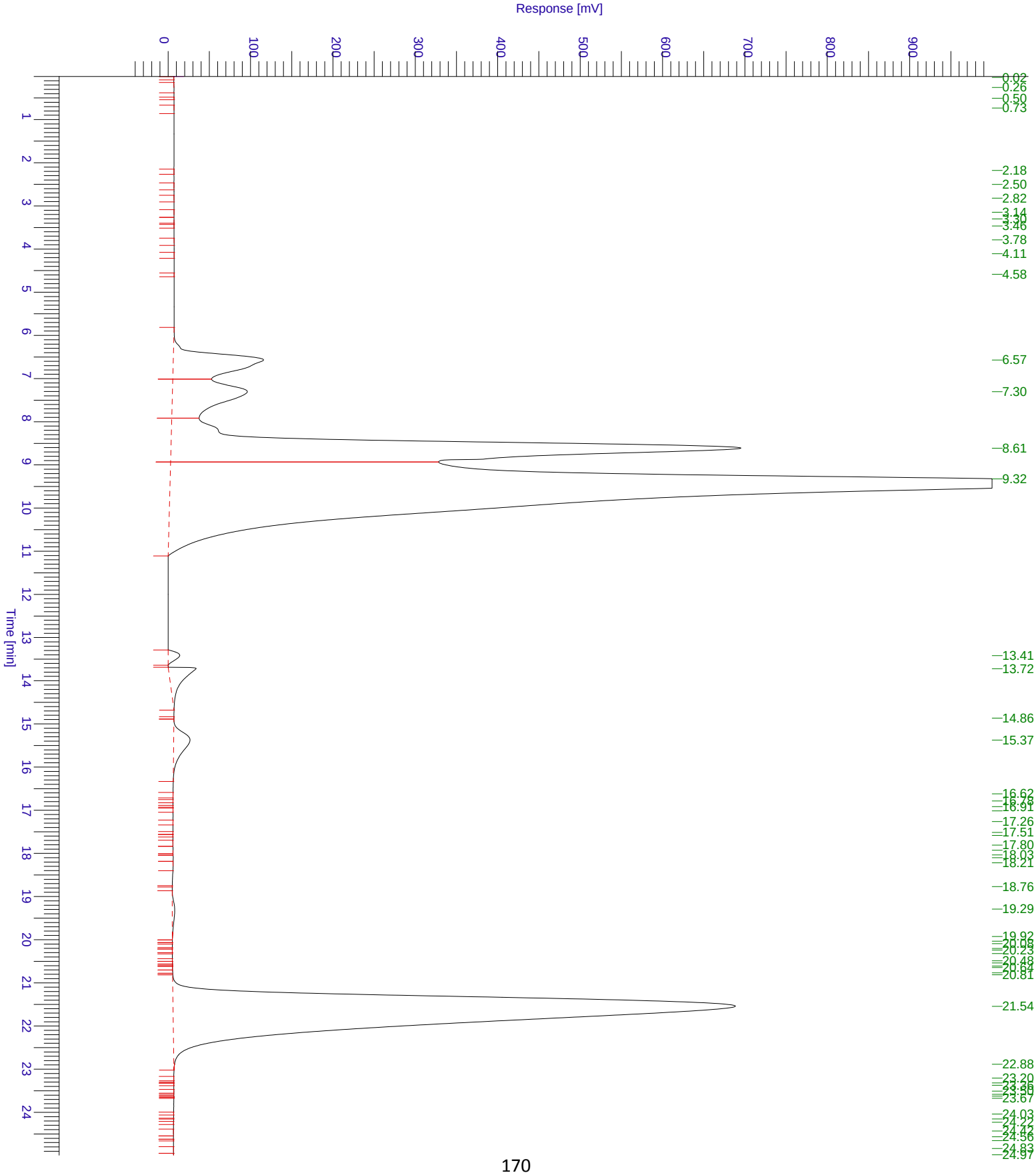
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S10 Sample #: 017 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol017.raw
Date : 6/21/2013 12:58:17 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 8:20:13 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -50.00 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -50.00 mV Plot Scale: 1050.0 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:51:08 PM
Operator	: rLuong	Sample Name	: S2
Sample Number	: 009	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/9
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 4:50:06 PM	Dilution Factor	: 1.00
		Cycle	: 9

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol009.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol009.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol009.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol009.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol009.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.196	132.80	53.94	0.00	0.00	BB	2.4622
2	0.470	148.20	73.60	0.00	0.00	BB	2.0136
3	0.616	123.20	58.11	0.00	0.00	BB	2.1199
4	0.884	222.00	62.81	0.00	0.00	BB	3.5342
5	1.033	524.34	90.07	0.00	0.00	BV	5.8215
6	1.204	558.08	85.80	0.00	0.00	VV	6.5044
7	1.370	170.78	66.04	0.00	0.00	VB	2.5859
8	1.618	151.80	64.94	0.00	0.00	BB	2.3375
9	1.769	440.80	58.03	0.00	0.00	BB	7.5965
10	1.923	186.00	93.92	0.00	0.00	BB	1.9805
11	2.351	184.00	60.86	0.00	0.00	BB	3.0231
12	2.715	68.40	66.70	6e-05	6e-05	BB	1.0254
13	2.803	167.60	49.92	0.00	0.00	BB	3.3573
14	3.255	151.22	74.26	0.00	0.00	BV	2.0363
15	3.295	309.58	74.78	0.00	0.00	VB	4.1397
16	3.453	359.60	81.15	0.00	0.00	BB	4.4313
17	3.646	396.80	76.80	0.00	0.00	BB	5.1668
18	4.062	62.20	44.30	5e-05	5e-05	BB	1.4042
19	4.424	86.00	42.19	7e-05	7e-05	BB	2.0382
20	4.756	213.60	88.53	0.00	0.00	BB	2.4127
21	5.386	100.20	38.02	9e-05	9e-05	BB	2.6355
22	5.472	116.00	69.26	1e-04	1e-04	BB	1.6749
23	5.703	51.00	43.55	4e-05	4e-05	BB	1.1710
24	6.263	37642.49	5077.80	0.03	0.03	BV	7.4131
25	6.594	5922086.07	242086.60	5.05	5.05	VV	24.4627
26	7.304	3660796.23	100751.52	3.12	3.12	VV	36.3349
27	8.195	948288.87	63714.40	0.81	0.81	VV	14.8834
28	8.611	15108230.12	639383.55	12.89	12.89	VV	23.6294
29	9.273	57923422.62	961605.23	49.41	49.41	VE	60.2362
30	11.757	492638.40	10507.86	0.42	0.42	EB	46.8828
31	13.088	593.22	142.16	0.00	0.00	BV	4.1730
32	13.401	63354.38	2573.13	0.05	0.05	VB	24.6215
33	14.369	445.80	87.21	0.00	0.00	BB	5.1120
34	15.376	130015.00	4480.87	0.11	0.11	BB	29.0156
35	16.698	185.20	58.88	0.00	0.00	BB	3.1452
36	17.438	138.40	56.88	0.00	0.00	BB	2.4332
37	18.306	9123.60	274.10	0.01	0.01	BB	33.2862
38	19.253	266.40	51.03	0.00	0.00	BB	5.2202
39	20.408	1098.53	129.09	0.00	0.00	BV	8.5097
40	20.540	925.59	168.73	0.00	0.00	VV	5.4855
41	21.543	32922225.82	819672.06	28.08	28.08	VB	40.1651
42	23.366	179.20	88.03	0.00	0.00	BB	2.0358
43	23.518	318.80	79.63	0.00	0.00	BB	4.0035
44	23.650	507.20	74.42	0.00	0.00	BB	6.8149
45	24.013	201.52	93.35	0.00	0.00	BV	2.1586

6/21/2013 12:51:08 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol009.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	24.070	193.08	71.76	0.00	0.00	VB	2.6908
47	24.226	125.00	60.99	0.00	0.00	BB	2.0496
48	24.667	324.80	76.91	0.00	0.00	BB	4.2229
49	24.824	125.60	63.98	0.00	0.00	BB	1.9632
50	24.976	50.80	36.68	4e-05	4e-05	BB	1.3851
		1.17e+08	2.85e+06	100.00	100.00		

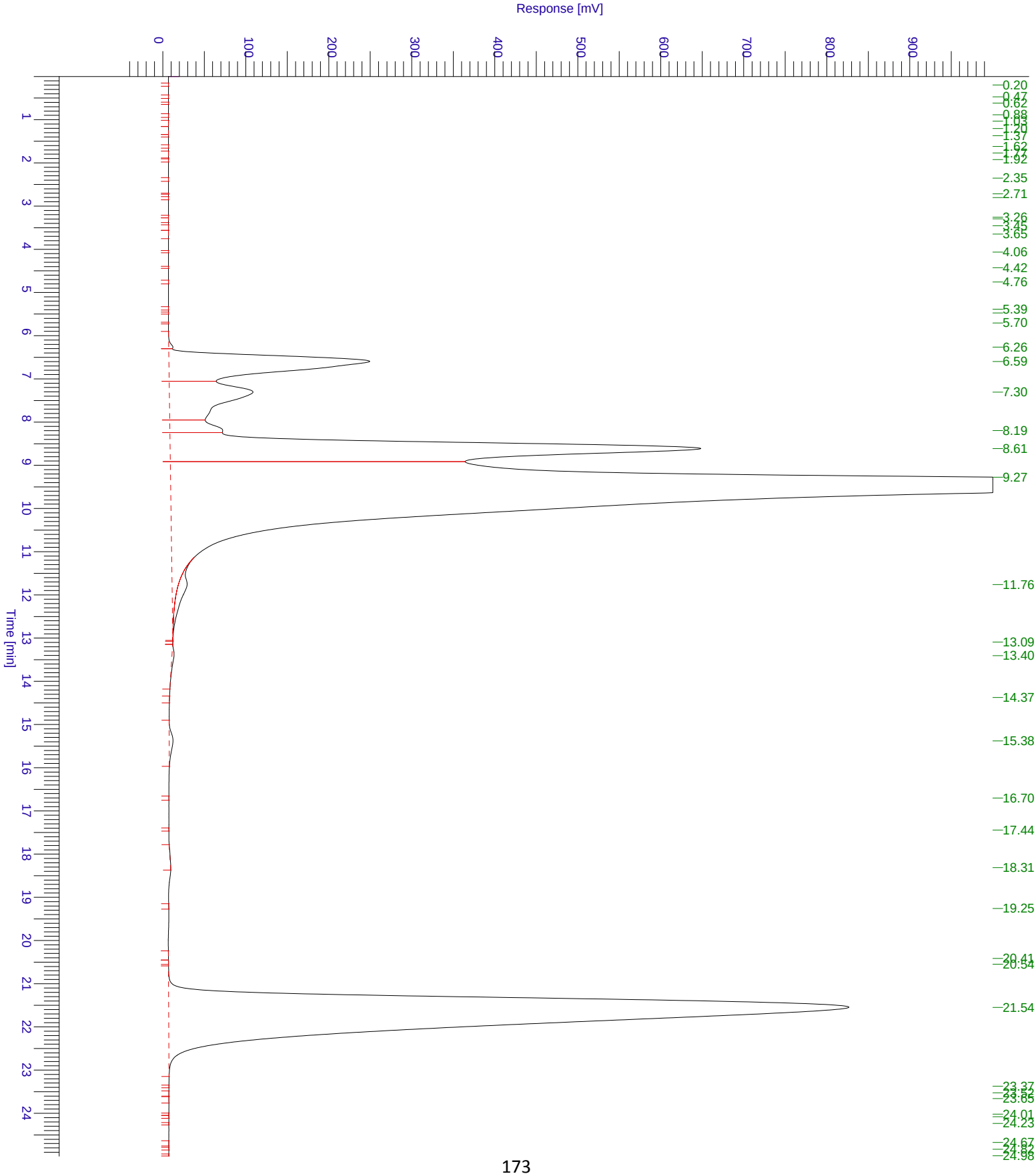
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S2 Sample #: 009 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol009.raw
Date : 6/21/2013 12:51:30 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 4:50:06 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -43.12 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -43.12 mV Plot Scale: 1043.1 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:51:55 PM
Operator	: rLuong	Sample Name	: S3
Sample Number	: 010	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/10
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 5:16:22 PM	Dilution Factor	: 1.00
		Cycle	: 10

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol010.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol010.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol010.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol010.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol010.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.193	265.20	77.33	0.00	0.00	BB	3.4295
2	0.312	140.40	78.44	0.00	0.00	BB	1.7899
3	0.480	368.00	105.95	0.00	0.00	BB	3.4735
4	0.561	54.20	46.01	5e-05	5e-05	BB	1.1780
5	0.647	215.60	55.33	0.00	0.00	BB	3.8969
6	0.768	91.47	82.44	8e-05	8e-05	BV	1.1096
7	0.824	264.33	79.48	0.00	0.00	VB	3.3257
8	1.013	140.20	56.55	0.00	0.00	BB	2.4792
9	1.630	390.40	93.68	0.00	0.00	BB	4.1675
10	1.762	126.00	54.84	0.00	0.00	BB	2.2975
11	1.838	194.00	68.06	0.00	0.00	BB	2.8504
12	2.074	493.80	109.35	0.00	0.00	BB	4.5159
13	2.326	385.70	57.08	0.00	0.00	BV	6.7576
14	2.404	359.90	92.18	0.00	0.00	VB	3.9044
15	2.570	105.20	55.68	9e-05	9e-05	BB	1.8895
16	2.720	468.00	50.52	0.00	0.00	BB	9.2646
17	3.048	172.80	72.57	0.00	0.00	BB	2.3811
18	4.164	79.00	39.39	7e-05	7e-05	BB	2.0055
19	4.349	115.20	50.25	0.00	0.00	BB	2.2925
20	5.463	44.60	42.31	4e-05	4e-05	BB	1.0541
21	6.254	36847.27	4874.22	0.03	0.03	BV	7.5596
22	6.581	5524360.03	227711.62	4.94	4.94	VV	24.2603
23	7.292	3298351.72	95081.92	2.95	2.95	VV	34.6896
24	8.187	897875.38	58892.62	0.80	0.80	VV	15.2460
25	8.597	14275177.86	612837.28	12.75	12.75	VV	23.2936
26	9.280	55726091.74	991821.24	49.79	49.79	VE	56.1856
27	11.710	345557.60	7798.38	0.31	0.31	EB	44.3114
28	13.001	501.16	124.66	0.00	0.00	BV	4.0202
29	13.392	385057.64	13678.16	0.34	0.34	VB	28.1513
30	14.770	187.93	73.55	0.00	0.00	BV	2.5550
31	15.362	365650.26	11778.82	0.33	0.33	VB	31.0430
32	16.857	312.40	53.36	0.00	0.00	BB	5.8549
33	17.189	195.00	78.79	0.00	0.00	BB	2.4750
34	17.357	152.51	56.68	0.00	0.00	BV	2.6905
35	18.308	81863.23	2136.11	0.07	0.07	VB	38.3236
36	18.895	193.01	90.51	0.00	0.00	BV	2.1325
37	19.239	14003.58	1473.50	0.01	0.01	VV	9.5036
38	19.298	11977.44	1564.08	0.01	0.01	VV	7.6578
39	19.392	25278.17	1531.96	0.02	0.02	VB	16.5006
40	19.962	98.40	41.23	9e-05	9e-05	BB	2.3866
41	20.021	111.40	48.79	1e-04	1e-04	BB	2.2835
42	20.140	63.60	55.08	6e-05	6e-05	BB	1.1547
43	20.238	356.32	79.98	0.00	0.00	BV	4.4552
44	20.387	465.73	78.03	0.00	0.00	VV	5.9686
45	20.486	263.33	74.55	0.00	0.00	VV	3.5323

6/21/2013 12:51:55 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol010.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	20.603	205.82	14.58	0.00	0.00	VB	14.1177
47	20.721	166.98	85.82	0.00	0.00	BV	1.9456
48	21.531	30922998.22	774537.66	27.63	27.63	VB	39.9245
49	23.451	166.60	79.06	0.00	0.00	BB	2.1073
50	23.493	280.80	99.53	0.00	0.00	BB	2.8213
51	23.710	138.80	51.20	0.00	0.00	BB	2.7109
52	23.900	45.77	52.73	4e-05	4e-05	BV	0.8679
53	23.931	372.63	131.75	0.00	0.00	VB	2.8283
54	24.105	115.60	112.55	0.00	0.00	BB	1.0271
55	24.168	68.40	52.57	6e-05	6e-05	BB	1.3012
56	24.261	263.20	55.97	0.00	0.00	BB	4.7026
57	24.439	500.20	93.43	0.00	0.00	BB	5.3537
58	24.615	67.76	67.13	6e-05	6e-05	BV	1.0094
59	24.641	54.72	67.06	5e-05	5e-05	VV	0.8160
60	24.739	552.43	84.68	0.00	0.00	VB	6.5236
61	24.909	230.80	110.69	0.00	0.00	BB	2.0851
		1.12e+08	2.81e+06	100.00	100.00		

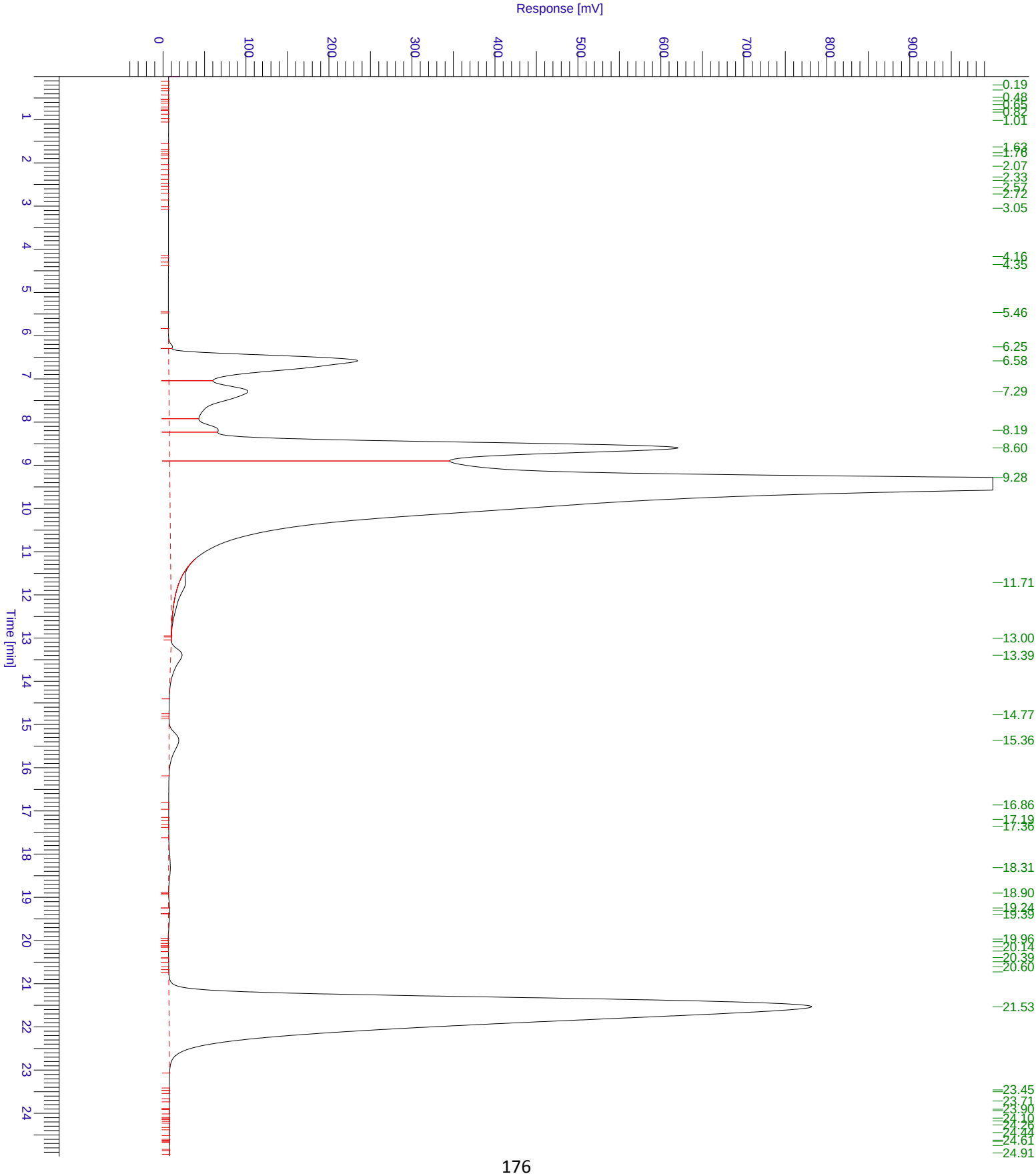
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S3 Sample #: 010 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol010.raw
Date : 6/21/2013 12:52:25 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 5:16:22 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -43.39 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -43.39 mV Plot Scale: 1043.4 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:53:00 PM
Operator	: rLuong	Sample Name	: S4
Sample Number	: 011	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/11
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 5:42:36 PM	Dilution Factor	: 1.00
		Cycle	: 11

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol011.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol011.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol011.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol011.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol011.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.277	1279.20	111.38	0.00	0.00	BB	11.4850
2	0.595	125.00	33.53	0.00	0.00	BB	3.7278
3	1.085	177.20	39.22	0.00	0.00	BB	4.5184
4	1.555	212.90	38.52	0.00	0.00	BV	5.5267
5	1.719	378.99	60.64	0.00	0.00	VV	6.2501
6	1.876	281.10	59.66	0.00	0.00	VB	4.7115
7	2.733	84.00	51.26	7e-05	7e-05	BB	1.6387
8	2.990	321.00	84.53	0.00	0.00	BB	3.7974
9	3.319	286.00	46.83	0.00	0.00	BB	6.1077
10	3.502	253.20	81.27	0.00	0.00	BB	3.1155
11	4.448	190.80	49.03	0.00	0.00	BB	3.8918
12	4.761	58.40	28.41	5e-05	5e-05	BB	2.0557
13	5.080	218.60	78.00	0.00	0.00	BB	2.8024
14	5.729	225.00	53.72	0.00	0.00	BB	4.1881
15	5.874	168.91	61.74	0.00	0.00	BV	2.7359
16	6.255	39932.78	5272.36	0.03	0.03	VV	7.5740
17	6.587	5938679.47	243956.29	4.97	4.97	VV	24.3432
18	7.294	3415176.78	102848.45	2.86	2.86	VV	33.2059
19	8.193	970817.25	63016.24	0.81	0.81	VV	15.4058
20	8.599	15232968.24	650597.16	12.75	12.75	VV	23.4138
21	9.293	57273847.59	993678.72	47.94	47.94	VE	57.6382
22	11.678	625340.40	9031.73	0.52	0.52	EV	69.2381
23	13.394	1813674.18	54228.32	1.52	1.52	VB	33.4451
24	14.714	152.80	95.09	0.00	0.00	BB	1.6069
25	14.780	196.73	65.54	0.00	0.00	BV	3.0016
26	14.882	155.78	79.94	0.00	0.00	VV	1.9487
27	15.364	633974.69	20336.52	0.53	0.53	VB	31.1742
28	16.369	59.60	70.33	5e-05	5e-05	BB	0.8474
29	16.408	93.60	58.75	8e-05	8e-05	BB	1.5931
30	16.648	121.80	74.67	0.00	0.00	BB	1.6312
31	16.711	257.40	92.72	0.00	0.00	BB	2.7761
32	16.802	56.40	38.57	5e-05	5e-05	BB	1.4621
33	16.868	122.00	80.39	0.00	0.00	BB	1.5175
34	17.135	109.77	88.44	9e-05	9e-05	BV	1.2411
35	17.203	266.23	79.23	0.00	0.00	VB	3.3603
36	17.281	113.80	77.95	1e-04	1e-04	BB	1.4598
37	17.382	242.52	66.99	0.00	0.00	BV	3.6201
38	17.460	100.08	63.59	8e-05	8e-05	VB	1.5740
39	17.573	228.60	98.86	0.00	0.00	BB	2.3123
40	17.692	151.60	51.19	0.00	0.00	BB	2.9617
41	17.770	114.80	73.00	1e-04	1e-04	BB	1.5727
42	17.889	82.80	76.26	7e-05	7e-05	BB	1.0858
43	17.922	53.80	53.04	5e-05	5e-05	BB	1.0142
44	17.998	72.70	91.45	6e-05	6e-05	BV	0.7950
45	18.087	945.04	212.55	0.00	0.00	VV	4.4462

6/21/2013 12:53:00 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol011.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	18.143	904.81	324.20	0.00	0.00	VV	2.7909
47	18.185	1059.79	312.83	0.00	0.00	VV	3.3877
48	18.252	914.85	278.48	0.00	0.00	VB	3.2852
49	18.533	330.00	93.06	0.00	0.00	BB	3.5460
50	19.305	6465.80	180.23	0.01	0.01	BB	35.8758
51	19.981	366.40	127.66	0.00	0.00	BB	2.8700
52	20.232	642.94	133.30	0.00	0.00	BV	4.8231
53	20.348	614.11	109.68	0.00	0.00	VV	5.5990
54	20.377	84.03	86.18	7e-05	7e-05	VV	0.9751
55	20.411	510.52	135.37	0.00	0.00	VB	3.7713
56	20.669	660.75	186.68	0.00	0.00	BV	3.5394
57	21.523	33503603.93	833111.27	28.04	28.04	VB	40.2150
58	23.396	54.80	57.43	5e-05	5e-05	BB	0.9542
59	23.488	432.39	111.32	0.00	0.00	BV	3.8842
60	23.559	1615.61	149.68	0.00	0.00	VB	10.7939
		1.19e+08	2.98e+06	100.00	100.00		

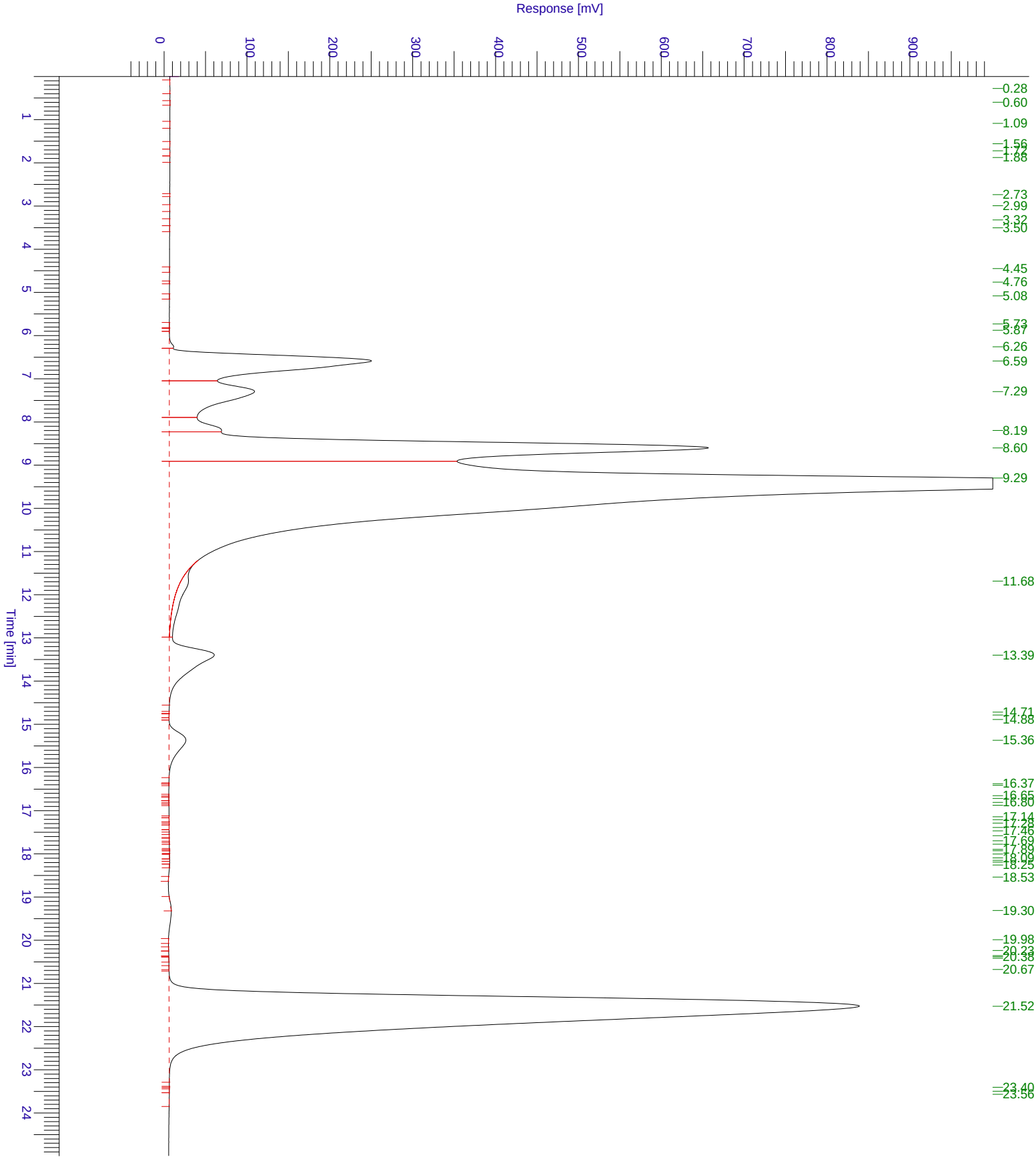
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S4 Sample #: 011 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol011.raw
Date : 6/21/2013 12:53:22 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 5:42:36 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -44.72 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -44.72 mV Plot Scale: 1044.7 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:53:44 PM
Operator	: rLuong	Sample Name	: S5
Sample Number	: 012	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/12
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 6:08:52 PM	Dilution Factor	: 1.00
		Cycle	: 12

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol012.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol012.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol012.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol012.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol012.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.220	495.20	84.97	0.00	0.00	BB	5.8282
2	0.302	38.80	37.84	4e-05	4e-05	BB	1.0254
3	0.500	54.00	48.93	5e-05	5e-05	BB	1.1036
4	0.683	100.40	49.74	1e-04	1e-04	BB	2.0184
5	0.827	57.00	58.10	5e-05	5e-05	BB	0.9811
6	0.975	126.00	107.08	0.00	0.00	BB	1.1767
7	1.007	68.00	68.57	6e-05	6e-05	BB	0.9917
8	1.281	243.60	82.57	0.00	0.00	BB	2.9501
9	1.416	442.60	101.22	0.00	0.00	BB	4.3727
10	1.544	223.60	88.76	0.00	0.00	BB	2.5191
11	1.610	55.60	78.04	5e-05	5e-05	BB	0.7125
12	1.646	653.40	104.37	0.00	0.00	BB	6.2602
13	1.836	196.00	88.27	0.00	0.00	BB	2.2204
14	1.905	204.81	129.94	0.00	0.00	BV	1.5762
15	1.933	306.92	129.70	0.00	0.00	VV	2.3663
16	1.992	89.47	77.96	8e-05	8e-05	VB	1.1477
17	2.036	158.40	66.40	0.00	0.00	BB	2.3855
18	2.120	96.40	97.39	9e-05	9e-05	BB	0.9898
19	2.154	339.20	94.22	0.00	0.00	BB	3.6000
20	2.446	493.43	140.07	0.00	0.00	BV	3.5228
21	2.507	90.57	85.51	9e-05	9e-05	VB	1.0592
22	2.549	169.00	101.31	0.00	0.00	BB	1.6682
23	2.766	148.20	96.98	0.00	0.00	BB	1.5281
24	2.827	463.60	100.71	0.00	0.00	BB	4.6034
25	2.905	65.00	67.72	6e-05	6e-05	BB	0.9599
26	3.028	72.60	79.26	7e-05	7e-05	BB	0.9160
27	3.073	144.40	64.84	0.00	0.00	BB	2.2271
28	3.158	64.00	64.56	6e-05	6e-05	BB	0.9913
29	3.227	458.40	108.36	0.00	0.00	BB	4.2302
30	3.354	58.00	59.07	5e-05	5e-05	BB	0.9819
31	3.387	109.40	57.83	0.00	0.00	BB	1.8918
32	3.571	258.80	88.90	0.00	0.00	BB	2.9113
33	3.714	814.60	150.27	0.00	0.00	BB	5.4211
34	3.842	328.00	108.07	0.00	0.00	BB	3.0351
35	3.971	240.40	97.44	0.00	0.00	BB	2.4672
36	4.120	40.40	35.79	4e-05	4e-05	BB	1.1289
37	4.380	150.62	90.11	0.00	0.00	BV	1.6716
38	4.415	253.58	83.10	0.00	0.00	VB	3.0516
39	4.552	38.40	52.14	4e-05	4e-05	BB	0.7364
40	4.713	218.20	71.67	0.00	0.00	BB	3.0443
41	4.857	202.40	73.75	0.00	0.00	BB	2.7446
42	5.018	436.00	82.95	0.00	0.00	BB	5.2560
43	5.170	112.80	47.57	0.00	0.00	BB	2.3712
44	5.490	506.40	98.89	0.00	0.00	BB	5.1207
45	5.668	210.60	71.02	0.00	0.00	BB	2.9654

6/21/2013 12:53:44 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol012.rst

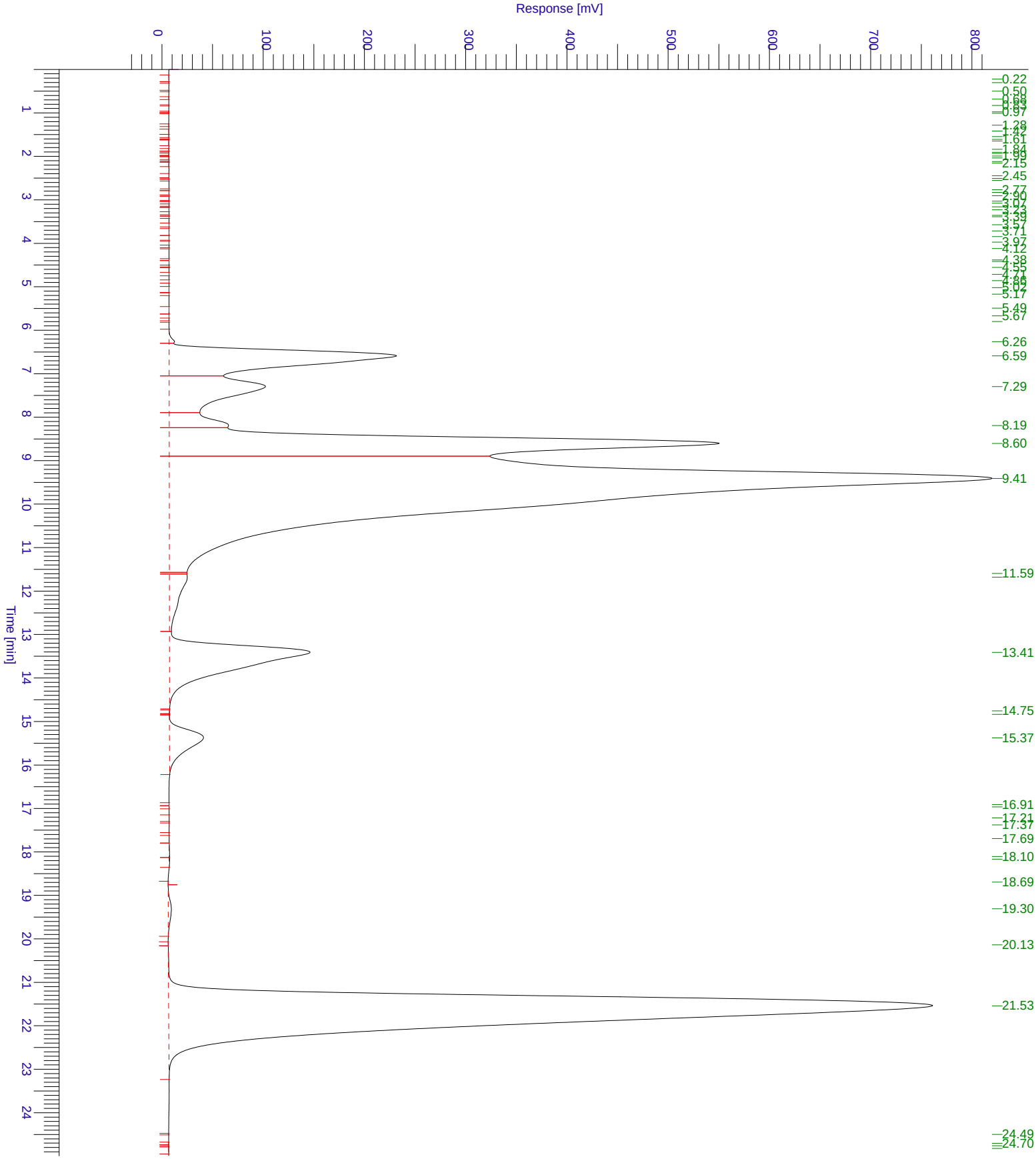
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	5.795	73.20	62.35	7e-05	7e-05	BB	1.1741
47	6.260	39588.99	5262.68	0.04	0.04	BV	7.5226
48	6.588	5460282.52	224549.54	5.17	5.17	VV	24.3166
49	7.292	3088469.08	95122.43	2.92	2.92	VV	32.4684
50	8.189	939755.31	58582.35	0.89	0.89	VV	16.0416
51	8.600	12805995.97	543270.64	12.12	12.12	VV	23.5720
52	9.405	46562236.85	812753.17	44.06	44.06	VV	57.2895
53	11.590	38911.10	17505.51	0.04	0.04	VV	2.2228
54	11.677	709439.53	17614.04	0.67	0.67	VV	40.2769
55	13.406	4757173.64	138832.55	4.50	4.50	VB	34.2656
56	14.749	291.60	104.86	0.00	0.00	BB	2.7808
57	14.833	75.80	76.87	7e-05	7e-05	BB	0.9861
58	15.370	1046583.00	33608.38	0.99	0.99	BB	31.1405
59	16.905	153.78	62.83	0.00	0.00	BV	2.4477
60	16.958	99.82	48.42	9e-05	9e-05	VB	2.0613
61	17.213	329.20	67.37	0.00	0.00	BB	4.8865
62	17.373	505.20	101.74	0.00	0.00	BB	4.9657
63	17.687	536.14	67.90	0.00	0.00	BV	7.8958
64	18.101	5653.23	433.31	0.01	0.01	VV	13.0467
65	18.160	4316.43	462.05	0.00	0.00	VB	9.3418
66	18.688	91.57	55.83	9e-05	9e-05	BV	1.6402
67	19.302	101185.22	3075.21	0.10	0.10	VB	32.9035
68	20.133	306.10	76.18	0.00	0.00	BV	4.0183
69	21.534	30105518.70	754596.23	28.49	28.49	VB	39.8962
70	24.492	72.80	59.04	7e-05	7e-05	BB	1.2330
71	24.696	124.80	51.87	0.00	0.00	BV	2.4062
72	24.752	107.40	93.43	0.00	0.00	VB	1.1495
73	24.814	357.20	83.17	0.00	0.00	BB	4.2949
		1.06e+08	2.71e+06	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S5 Sample #: 012 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol012.raw
Date : 6/21/2013 12:54:10 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 6:08:52 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -34.59 mV High Point : 819.95 mV
Scale Factor: 1.0 Plot Offset: -34.59 mV Plot Scale: 854.5 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:54:42 PM
Operator	: rLuong	Sample Name	: S6
Sample Number	: 013	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/13
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 6:35:09 PM	Dilution Factor	: 1.00
		Cycle	: 13

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol013.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol013.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol013.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol013.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol013.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.103	177.20	86.26	0.00	0.00	BB	2.0543
2	0.192	349.58	91.76	0.00	0.00	BV	3.8095
3	0.270	189.22	48.21	0.00	0.00	VB	3.9253
4	0.325	60.00	47.00	6e-05	6e-05	BB	1.2767
5	0.435	286.00	76.58	0.00	0.00	BB	3.7345
6	0.845	546.80	94.48	0.00	0.00	BB	5.7874
7	1.178	28.80	32.64	3e-05	3e-05	BB	0.8823
8	1.535	176.40	68.50	0.00	0.00	BB	2.5752
9	1.709	114.80	75.16	0.00	0.00	BB	1.5275
10	1.981	199.60	56.26	0.00	0.00	BB	3.5478
11	2.185	170.20	58.35	0.00	0.00	BB	2.9170
12	2.461	64.40	51.13	6e-05	6e-05	BB	1.2596
13	2.659	73.20	44.61	7e-05	7e-05	BB	1.6409
14	2.985	66.40	57.93	6e-05	6e-05	BB	1.1461
15	3.236	119.60	69.06	0.00	0.00	BB	1.7319
16	3.793	64.80	47.18	6e-05	6e-05	BB	1.3733
17	4.048	125.00	64.13	0.00	0.00	BB	1.9491
18	4.112	248.60	97.81	0.00	0.00	BB	2.5417
19	4.278	131.20	62.53	0.00	0.00	BB	2.0984
20	4.434	250.00	66.81	0.00	0.00	BB	3.7421
21	4.799	73.66	79.26	7e-05	7e-05	BV	0.9293
22	4.827	109.34	78.07	0.00	0.00	VB	1.4006
23	5.083	251.60	100.07	0.00	0.00	BB	2.5142
24	5.617	158.80	65.72	0.00	0.00	BB	2.4163
25	5.903	155.20	65.86	0.00	0.00	BB	2.3566
26	6.260	33235.55	4846.46	0.03	0.03	BV	6.8577
27	6.595	5852781.47	238206.53	5.48	5.48	VV	24.5702
28	7.294	3087462.57	98766.66	2.89	2.89	VV	31.2602
29	8.183	908835.28	56885.61	0.85	0.85	VV	15.9765
30	8.601	10426222.13	443359.96	9.76	9.76	VV	23.5164
31	9.437	40309021.43	562440.36	37.74	37.74	VV	71.6681
32	11.682	597.97	102.21	0.00	0.00	VB	5.8505
33	13.412	8713618.80	260862.36	8.16	8.16	BB	33.4031
34	15.374	2041794.80	64568.30	1.91	1.91	BB	31.6222
35	16.718	100.60	53.59	9e-05	9e-05	BB	1.8773
36	16.791	93.60	37.28	9e-05	9e-05	BB	2.5110
37	16.971	320.25	85.68	0.00	0.00	BV	3.7378
38	17.163	456.15	49.84	0.00	0.00	VB	9.1521
39	17.527	54.20	54.39	5e-05	5e-05	BB	0.9965
40	18.089	1374.75	103.35	0.00	0.00	BV	13.3015
41	18.141	224.05	90.82	0.00	0.00	VB	2.4669
42	18.736	339.46	86.19	0.00	0.00	BV	3.9385
43	19.324	110125.34	3379.56	0.10	0.10	VB	32.5857
44	21.520	35321525.60	874316.07	33.07	33.07	BB	40.3990
45	23.579	85.80	89.36	8e-05	8e-05	BB	0.9602

6/21/2013 12:54:42 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol013.rst

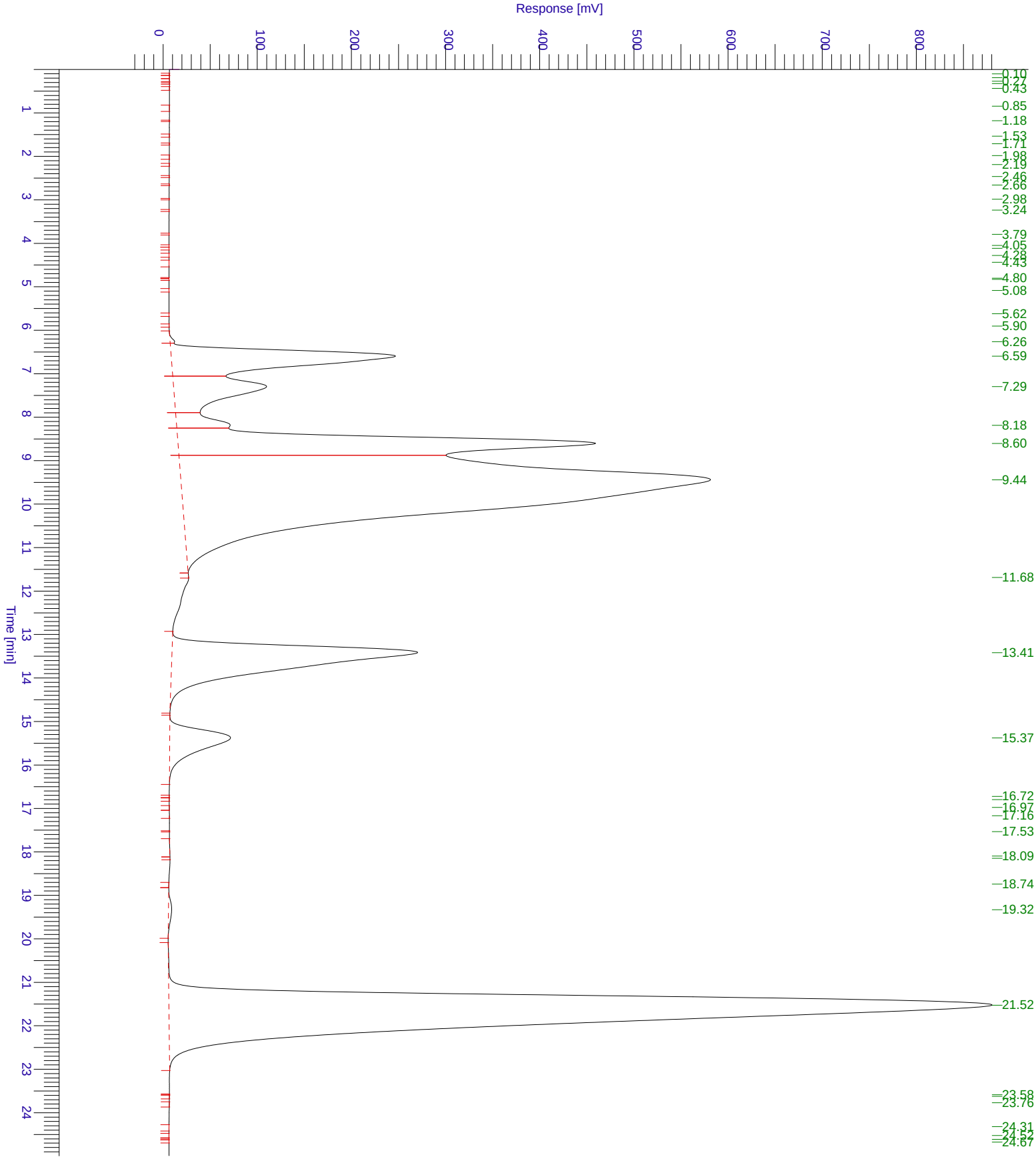
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	23.616	313.20	119.57	0.00	0.00	BB	2.6194
47	23.763	320.40	83.57	0.00	0.00	BB	3.8338
48	24.313	553.00	58.58	0.00	0.00	BB	9.4403
49	24.516	426.00	117.47	0.00	0.00	BB	3.6265
50	24.606	60.80	53.65	6e-05	6e-05	BB	1.1333
51	24.670	200.00	52.56	0.00	0.00	BB	3.8050
		1.07e+08	2.61e+06	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S6 Sample #: 013 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol013.raw
Date : 6/21/2013 12:55:09 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 6:35:09 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -38.41 mV High Point : 880.44 mV
Scale Factor: 1.0 Plot Offset: -38.41 mV Plot Scale: 918.8 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:55:33 PM
Operator	: rLuong	Sample Name	: S7
Sample Number	: 014	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/14
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 7:01:25 PM	Dilution Factor	: 1.00
		Cycle	: 14

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol014.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol014.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol014.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol014.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol014.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.182	701.94	102.73	0.00	0.00	BV	6.8329
2	0.257	202.46	90.41	0.00	0.00	VB	2.2393
3	0.429	248.80	57.23	0.00	0.00	BB	4.3472
4	0.758	44.00	59.83	4e-05	4e-05	BB	0.7354
5	0.819	57.20	44.50	5e-05	5e-05	BB	1.2853
6	1.458	332.27	73.85	0.00	0.00	BV	4.4991
7	1.550	172.13	58.53	0.00	0.00	VB	2.9410
8	1.628	291.40	95.88	0.00	0.00	BB	3.0392
9	1.817	342.60	85.27	0.00	0.00	BB	4.0179
10	2.010	283.20	50.20	0.00	0.00	BB	5.6411
11	2.436	434.80	126.67	0.00	0.00	BB	3.4326
12	2.581	233.80	61.13	0.00	0.00	BB	3.8248
13	2.760	498.80	76.80	0.00	0.00	BB	6.4946
14	2.895	227.20	75.14	0.00	0.00	BB	3.0236
15	3.226	130.80	57.57	0.00	0.00	BB	2.2720
16	3.413	115.20	51.21	0.00	0.00	BB	2.2497
17	3.554	394.80	78.98	0.00	0.00	BB	4.9989
18	4.035	207.60	98.84	0.00	0.00	BB	2.1004
19	4.707	125.80	71.18	0.00	0.00	BB	1.7673
20	5.020	251.60	72.92	0.00	0.00	BB	3.4505
21	5.343	335.60	94.22	0.00	0.00	BB	3.5620
22	5.627	170.77	66.82	0.00	0.00	BV	2.5557
23	5.689	230.13	70.59	0.00	0.00	VV	3.2599
24	5.807	209.32	67.74	0.00	0.00	VV	3.0901
25	6.570	3043072.04	108971.37	2.66	2.66	VV	27.9254
26	7.306	3391274.76	88556.87	2.97	2.97	VV	38.2949
27	8.610	17120747.91	694553.20	14.98	14.98	VV	24.6500
28	9.273	57860520.07	953423.30	50.62	50.62	VE	60.6871
29	11.670	225677.20	3600.14	0.20	0.20	EV	62.6856
30	13.400	142700.21	3659.55	0.12	0.12	VB	38.9940
31	15.345	43749.60	1519.06	0.04	0.04	BB	28.8004
32	16.221	126.00	51.80	0.00	0.00	BB	2.4326
33	16.382	95.80	51.42	8e-05	8e-05	BB	1.8629
34	16.709	227.60	73.43	0.00	0.00	BB	3.0996
35	16.879	370.42	71.87	0.00	0.00	BV	5.1543
36	17.039	209.38	76.21	0.00	0.00	VB	2.7475
37	17.387	91.20	29.39	8e-05	8e-05	BB	3.1034
38	17.752	626.05	137.12	0.00	0.00	BV	4.5658
39	17.935	2454.64	300.12	0.00	0.00	VV	8.1788
40	18.322	30133.30	1102.69	0.03	0.03	VB	27.3270
41	19.367	293.80	59.60	0.00	0.00	BB	4.9299
42	19.598	73.60	47.65	6e-05	6e-05	BB	1.5447
43	20.093	124.40	62.89	0.00	0.00	BB	1.9781
44	20.254	114.80	51.14	0.00	0.00	BB	2.2450
45	20.443	344.54	48.55	0.00	0.00	BV	7.0960

6/21/2013 12:55:33 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol014.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	21.530	32427737.46	809940.45	28.37	28.37	VB	40.0372
47	23.200	279.60	103.63	0.00	0.00	BB	2.6980
48	23.537	161.40	103.97	0.00	0.00	BB	1.5523
49	23.635	172.40	73.85	0.00	0.00	BB	2.3343
50	23.703	202.00	68.78	0.00	0.00	BB	2.9368
51	23.772	211.60	64.49	0.00	0.00	BB	3.2812
52	23.968	103.20	78.92	9e-05	9e-05	BB	1.3076
53	24.027	127.20	63.29	0.00	0.00	BB	2.0099
54	24.282	187.73	56.92	0.00	0.00	BV	3.2982
55	24.333	56.27	56.09	5e-05	5e-05	VB	1.0032
56	24.473	163.40	79.32	0.00	0.00	BB	2.0599
		1.14e+08	2.67e+06	100.00	100.00		

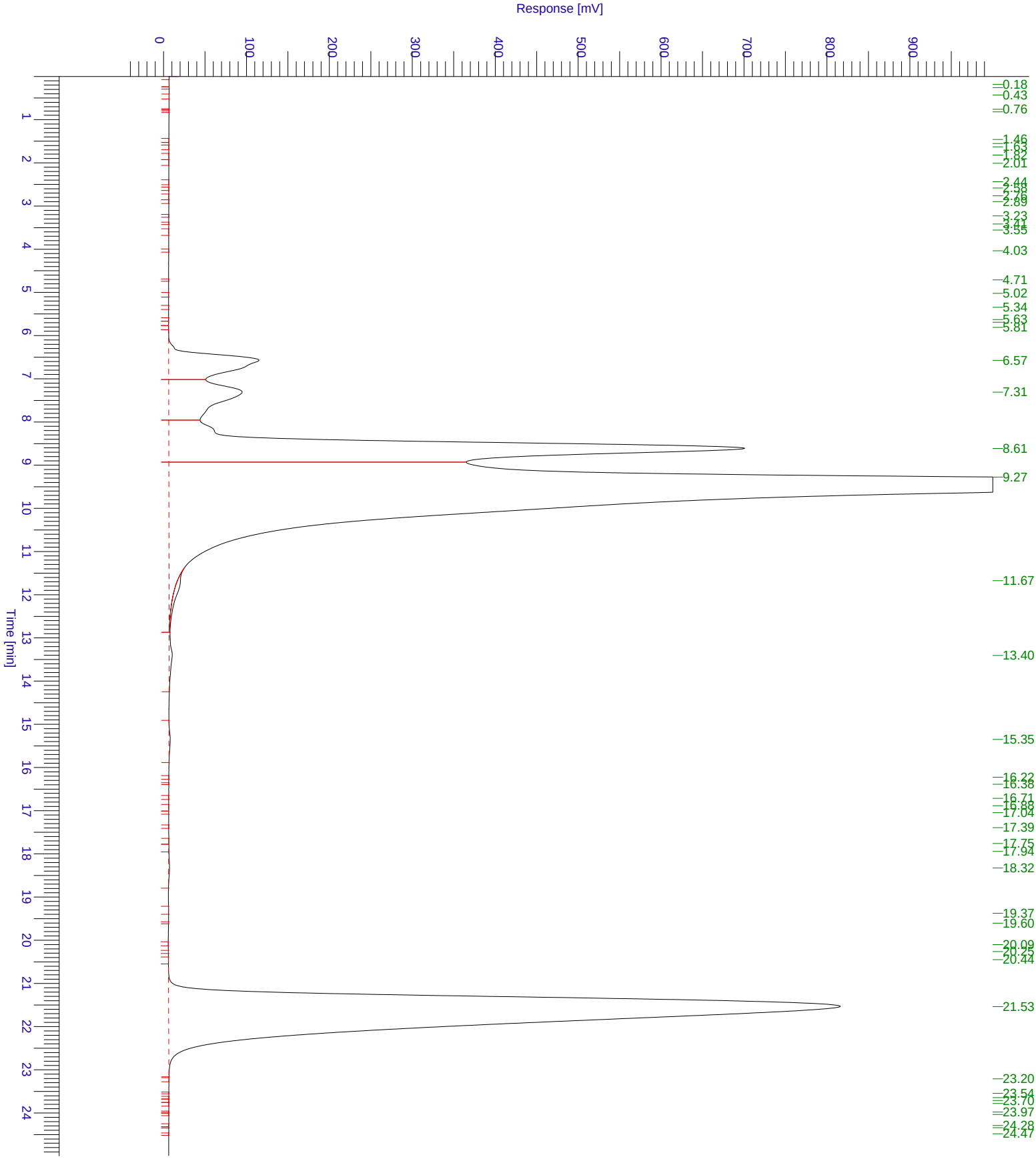
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S7 Sample #: 014 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol014.raw
Date : 6/21/2013 12:55:53 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 7:01:25 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -44.05 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -44.05 mV Plot Scale: 1044.1 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:16:12 PM
Operator	: rLuong	Sample Name	: S70
Sample Number	: 077	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/77
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/12/2013 10:38:29 PM	Dilution Factor	: 1.00
		Cycle	: 77

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol077.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol077.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol077.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol077.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol077.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

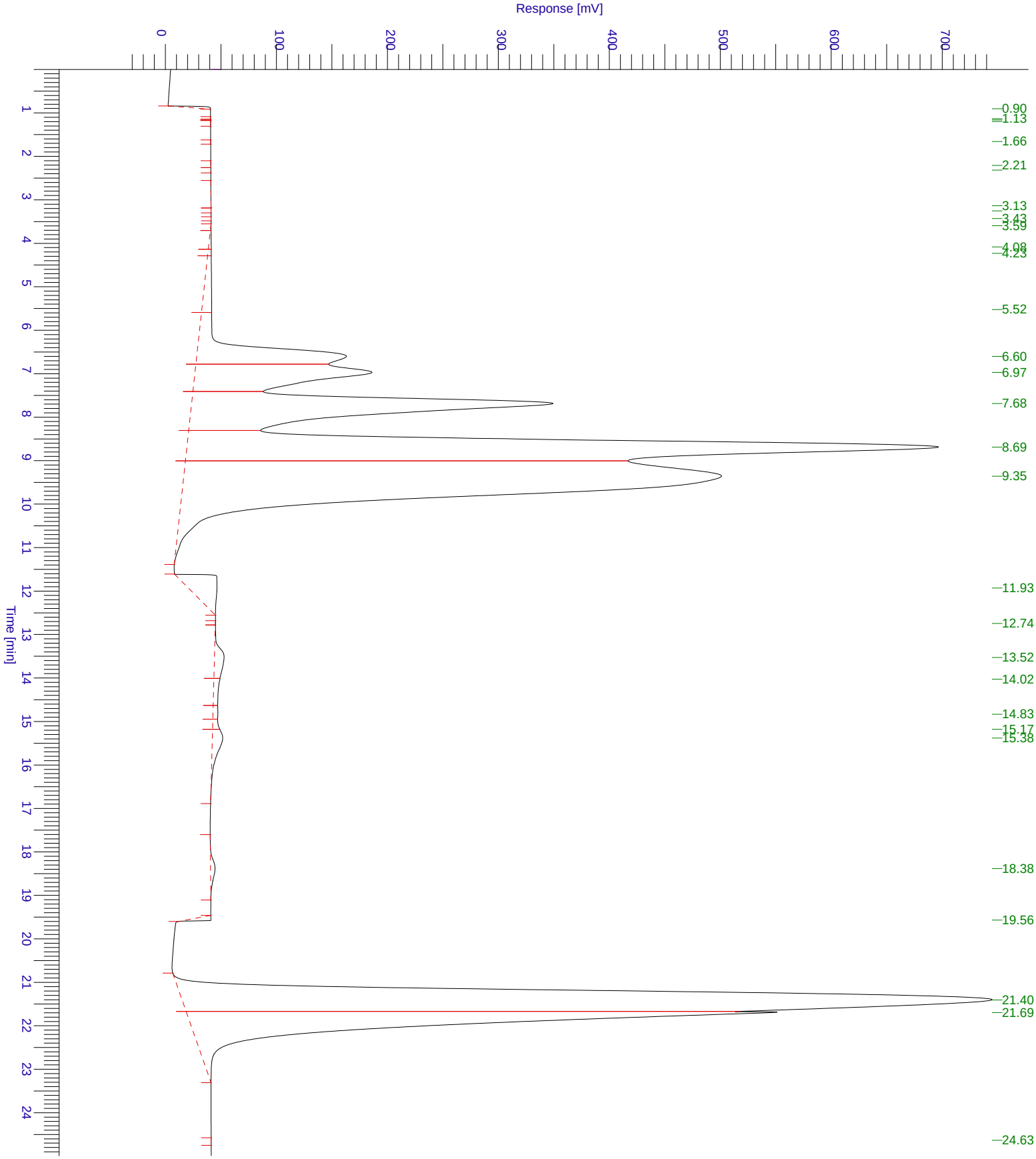
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.905	64556.80	4510.92	0.07	0.07	BB	14.3112
2	1.133	129.60	85.60	0.00	0.00	BB	1.5141
3	1.161	99.20	103.77	0.00	0.00	BB	0.9560
4	1.198	743.40	271.10	0.00	0.00	BB	2.7421
5	1.655	80.40	25.01	9e-05	9e-05	BB	3.2141
6	2.208	309.26	49.53	0.00	0.00	BV	6.2436
7	2.315	109.74	28.72	0.00	0.00	VB	3.8211
8	3.134	702.00	36.70	0.00	0.00	BB	19.1298
9	3.252	74.40	22.43	8e-05	8e-05	BB	3.3175
10	3.429	77.80	26.86	9e-05	9e-05	BB	2.8969
11	3.594	3140.82	201.05	0.00	0.00	BV	15.6224
12	4.080	42154.04	2358.38	0.05	0.05	VV	17.8741
13	4.227	26738.43	3011.27	0.03	0.03	VV	8.8794
14	5.522	487942.38	8894.10	0.53	0.53	VV	54.8613
15	6.600	3411935.02	134921.49	3.73	3.73	VV	25.2883
16	6.968	4341005.67	159498.19	4.74	4.74	VV	27.2166
17	7.684	8886628.65	325662.36	9.71	9.71	VV	27.2879
18	8.686	17339924.75	677301.41	18.95	18.95	VV	25.6015
19	9.354	24353455.84	484550.58	26.62	26.62	VB	50.2599
20	11.926	1065996.40	25816.32	1.17	1.17	BB	41.2916
21	12.741	509.97	118.04	0.00	0.00	BV	4.3204
22	13.524	339063.97	8639.88	0.37	0.37	VV	39.2441
23	14.023	161965.77	5497.75	0.18	0.18	VV	29.4604
24	14.829	82305.06	4481.02	0.09	0.09	VV	18.3675
25	15.174	70844.77	6582.37	0.08	0.08	VV	10.7628
26	15.376	340806.87	9337.87	0.37	0.37	VB	36.4973
27	18.376	130818.00	4068.31	0.14	0.14	BB	32.1553
28	19.562	100141.20	21427.21	0.11	0.11	BB	4.6736
29	21.399	19917628.98	730074.73	21.77	21.77	BV	27.2816
30	21.690	10316929.62	535417.64	11.28	11.28	VB	19.2689
31	24.627	111.20	24.86	0.00	0.00	BB	4.4733
		91486930.00	3.15e+06	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S70 Sample #: 077 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol077.raw
Date : 6/21/2013 2:16:37 PM
Method : Method Robin 87H Time of Injection: 6/12/2013 10:38:29 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -34.71 mV High Point : 744.99 mV
Scale Factor: 1.0 Plot Offset: -34.71 mV Plot Scale: 779.7 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:17:10 PM
Operator	: rLuong	Sample Name	: S71
Sample Number	: 078	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/78
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/12/2013 11:04:49 PM	Dilution Factor	: 1.00
		Cycle	: 78

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol078.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol078.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol078.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol078.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol078.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.038	959.20	277.17	0.00	0.00	BV	3.4607
2	0.953	235494.00	8337.34	0.26	0.26	VB	28.2457
3	3.485	38014.60	1649.01	0.04	0.04	BB	23.0530
4	4.083	443.04	115.48	0.00	0.00	BV	3.8365
5	4.155	439.16	182.14	0.00	0.00	VB	2.4110
6	4.244	528.05	130.15	0.00	0.00	BV	4.0573
7	4.309	284.75	187.55	0.00	0.00	VB	1.5182
8	4.393	430.92	123.86	0.00	0.00	BV	3.4791
9	4.460	432.28	220.77	0.00	0.00	VB	1.9580
10	4.549	496.43	133.99	0.00	0.00	BV	3.7049
11	4.619	471.97	241.75	0.00	0.00	VB	1.9523
12	4.709	618.78	157.25	0.00	0.00	BV	3.9351
13	4.784	584.42	276.85	0.00	0.00	VB	2.1109
14	4.871	509.59	124.55	0.00	0.00	BV	4.0916
15	4.953	591.61	250.10	0.00	0.00	VB	2.3655
16	5.048	342.38	99.00	0.00	0.00	BV	3.4584
17	5.130	515.02	190.89	0.00	0.00	VB	2.6980
18	5.498	3801.60	265.55	0.00	0.00	BV	14.3157
19	5.568	980.22	321.10	0.00	0.00	VV	3.0526
20	5.673	443.46	118.91	0.00	0.00	VV	3.7293
21	5.753	764.33	218.96	0.00	0.00	VB	3.4906
22	5.842	143.06	60.15	0.00	0.00	BV	2.3782
23	5.948	529.74	163.83	0.00	0.00	VB	3.2335
24	6.052	125.60	58.44	0.00	0.00	BB	2.1490
25	6.598	2637682.68	123433.63	2.96	2.96	BV	21.3692
26	6.970	3686392.74	142342.70	4.13	4.13	VV	25.8980
27	7.684	7786765.29	300833.37	8.73	8.73	VV	25.8840
28	8.689	17745862.99	700544.48	19.89	19.89	VV	25.3315
29	9.358	25240054.90	510220.58	28.29	28.29	VE	49.4689
30	11.300	15135.20	168.31	0.02	0.02	EB	89.9260
31	11.393	120.20	123.47	0.00	0.00	BB	0.9735
32	11.456	505.62	245.33	0.00	0.00	BV	2.0610
33	11.530	1534.15	514.85	0.00	0.00	VV	2.9798
34	11.584	1636.71	593.91	0.00	0.00	VV	2.7558
35	11.614	1216.80	694.73	0.00	0.00	VV	1.7515
36	11.664	2853.13	612.50	0.00	0.00	VB	4.6582
37	11.799	255.48	129.53	0.00	0.00	BV	1.9723
38	11.847	819.32	221.92	0.00	0.00	VB	3.6919
39	11.968	1302.24	436.64	0.00	0.00	BV	2.9824
40	12.025	1412.54	533.96	0.00	0.00	VV	2.6454
41	12.051	409.42	389.76	0.00	0.00	VB	1.0504
42	12.127	1239.01	463.23	0.00	0.00	BV	2.6747
43	12.186	2339.79	605.44	0.00	0.00	VV	3.8646
44	12.267	1825.66	499.91	0.00	0.00	VV	3.6520
45	12.310	853.96	564.87	0.00	0.00	VV	1.5118

6/21/2013 2:17:10 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol078.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	12.336	722.78	525.02	0.00	0.00	VV	1.3767
47	12.362	547.19	467.05	0.00	0.00	VB	1.1716
48	12.448	2196.12	511.49	0.00	0.00	BV	4.2936
49	12.503	1959.48	634.42	0.00	0.00	VB	3.0886
50	12.620	9327.05	2086.56	0.01	0.01	BV	4.4701
51	12.669	12688.08	3100.20	0.01	0.01	VV	4.0927
52	12.750	15461.37	4668.81	0.02	0.02	VV	3.3116
53	12.788	14903.60	5248.65	0.02	0.02	VV	2.8395
54	12.843	24219.99	6125.44	0.03	0.03	VV	3.9540
55	12.947	53367.86	7937.51	0.06	0.06	VV	6.7235
56	13.020	17774.72	9377.60	0.02	0.02	VV	1.8954
57	13.048	21250.75	9991.96	0.02	0.02	VV	2.1268
58	13.116	38647.81	11282.45	0.04	0.04	VV	3.4255
59	13.171	36195.55	12364.47	0.04	0.04	VV	2.9274
60	13.195	21603.14	12882.64	0.02	0.02	VV	1.6769
61	13.468	275907.99	20573.66	0.31	0.31	VV	13.4107
62	13.488	37407.27	20996.76	0.04	0.04	VV	1.7816
63	13.547	63388.63	22346.93	0.07	0.07	VB	2.8366
64	16.076	252983.80	16477.62	0.28	0.28	BB	15.3532
65	18.376	327955.20	10071.01	0.37	0.37	BE	32.5643
66	19.134	1139.60	203.37	0.00	0.00	EB	5.6035
67	20.154	605.60	79.11	0.00	0.00	BB	7.6549
68	20.378	525.20	66.76	0.00	0.00	BB	7.8674
69	20.616	1236.00	168.45	0.00	0.00	BB	7.3376
70	21.400	30560026.80	765124.23	34.25	34.25	MM	39.9413
71	24.788	5365.40	252.22	0.01	0.01	BB	21.2730
		89215569.00	2.74e+06	100.00	100.00		

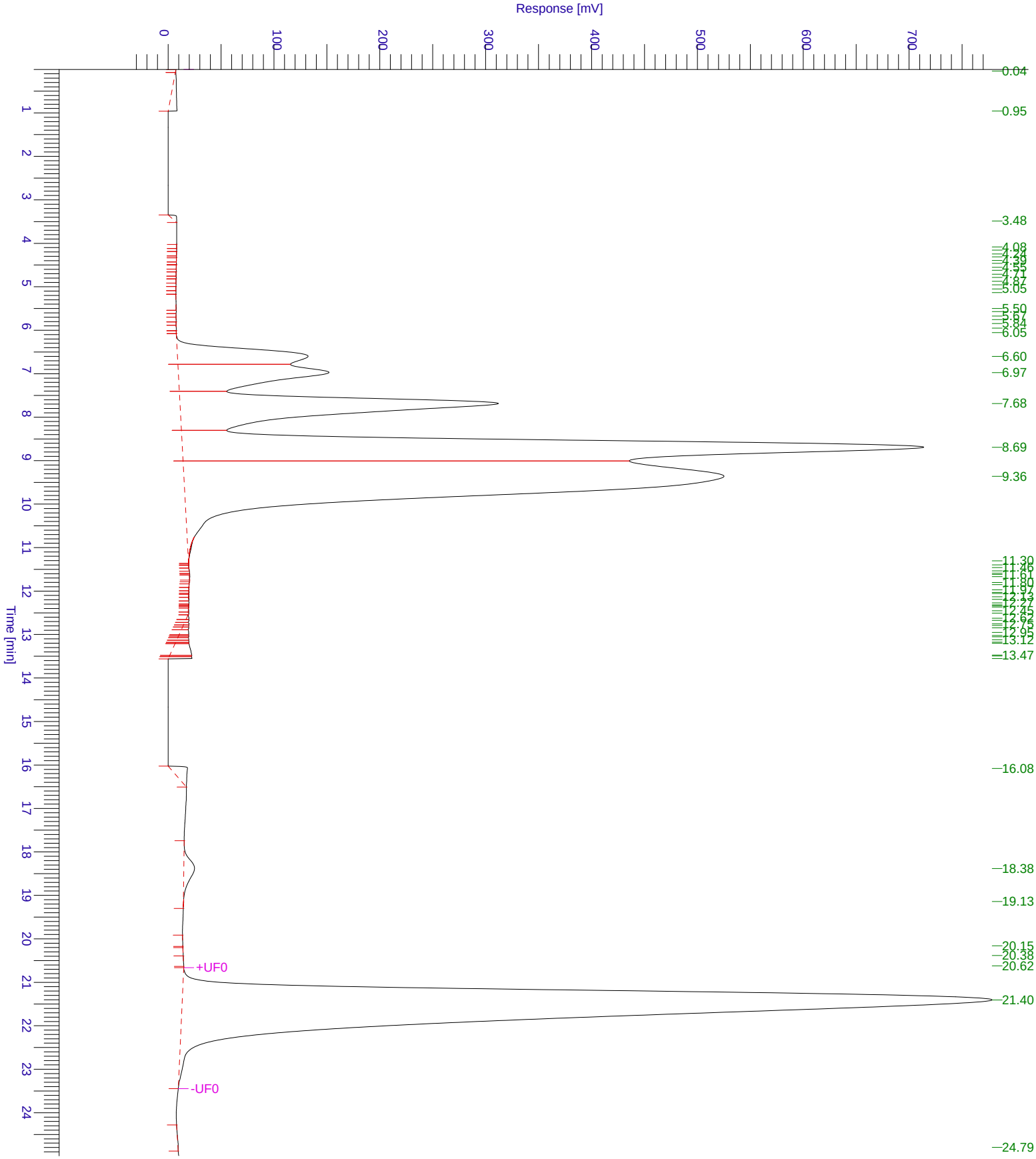
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S71 Sample #: 078 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol078.raw
Date : 6/21/2013 2:17:33 PM
Method : Method Robin 87H Time of Injection: 6/12/2013 11:04:49 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -38.92 mV High Point : 778.43 mV
Scale Factor: 1.0 Plot Offset: -38.92 mV Plot Scale: 817.4 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:18:06 PM
Operator	: rLuong	Sample Name	: S72
Sample Number	: 079	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/79
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/12/2013 11:31:09 PM	Dilution Factor	: 1.00
		Cycle	: 79

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol079.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol079.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol079.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol079.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol079.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	2.143	27121.60	1610.93	0.03	0.03	BB	16.8359
2	2.655	974.20	84.05	0.00	0.00	BB	11.5911
3	2.955	870.97	183.42	0.00	0.00	BV	4.7484
4	3.242	8502.74	496.40	0.01	0.01	VV	17.1287
5	3.558	9398.49	677.85	0.01	0.01	VV	13.8652
6	3.789	9170.13	938.69	0.01	0.01	VV	9.7691
7	3.959	13701.49	1216.20	0.02	0.02	VV	11.2658
8	4.259	40975.77	1398.93	0.05	0.05	VV	29.2908
9	5.966	247054.79	4442.06	0.30	0.30	VV	55.6172
10	6.600	2594203.64	117681.22	3.15	3.15	VV	22.0443
11	6.968	3546651.11	135885.84	4.31	4.31	VV	26.1002
12	7.684	7207074.37	282296.01	8.75	8.75	VV	25.5302
13	8.688	16946086.21	677999.26	20.58	20.58	VV	24.9943
14	9.355	24583375.40	501571.23	29.86	29.86	VV	49.0127
15	11.600	88125.43	6728.75	0.11	0.11	VB	13.0968
16	14.237	108731.60	7276.72	0.13	0.13	BB	14.9424
17	15.409	531828.20	16899.18	0.65	0.65	BB	31.4707
18	16.227	83.00	39.68	0.00	0.00	BB	2.0918
19	16.510	595.60	113.10	0.00	0.00	BB	5.2660
20	16.850	2420.26	180.88	0.00	0.00	BV	13.3806
21	16.959	999.74	138.70	0.00	0.00	VB	7.2080
22	17.251	188.40	32.06	0.00	0.00	BB	5.8770
23	17.479	280.40	72.14	0.00	0.00	BB	3.8871
24	17.901	35050.57	2304.94	0.04	0.04	BV	15.2067
25	18.273	173780.03	4380.68	0.21	0.21	VB	39.6696
26	20.342	4417.22	382.89	0.01	0.01	BV	11.5365
27	20.518	3478.58	309.45	0.00	0.00	VB	11.2410
28	21.400	26137263.62	693400.25	31.75	31.75	MM	37.6943
29	24.478	764.40	92.09	0.00	0.00	BB	8.3006
		82323167.96	2.46e+06	100.00	100.00		

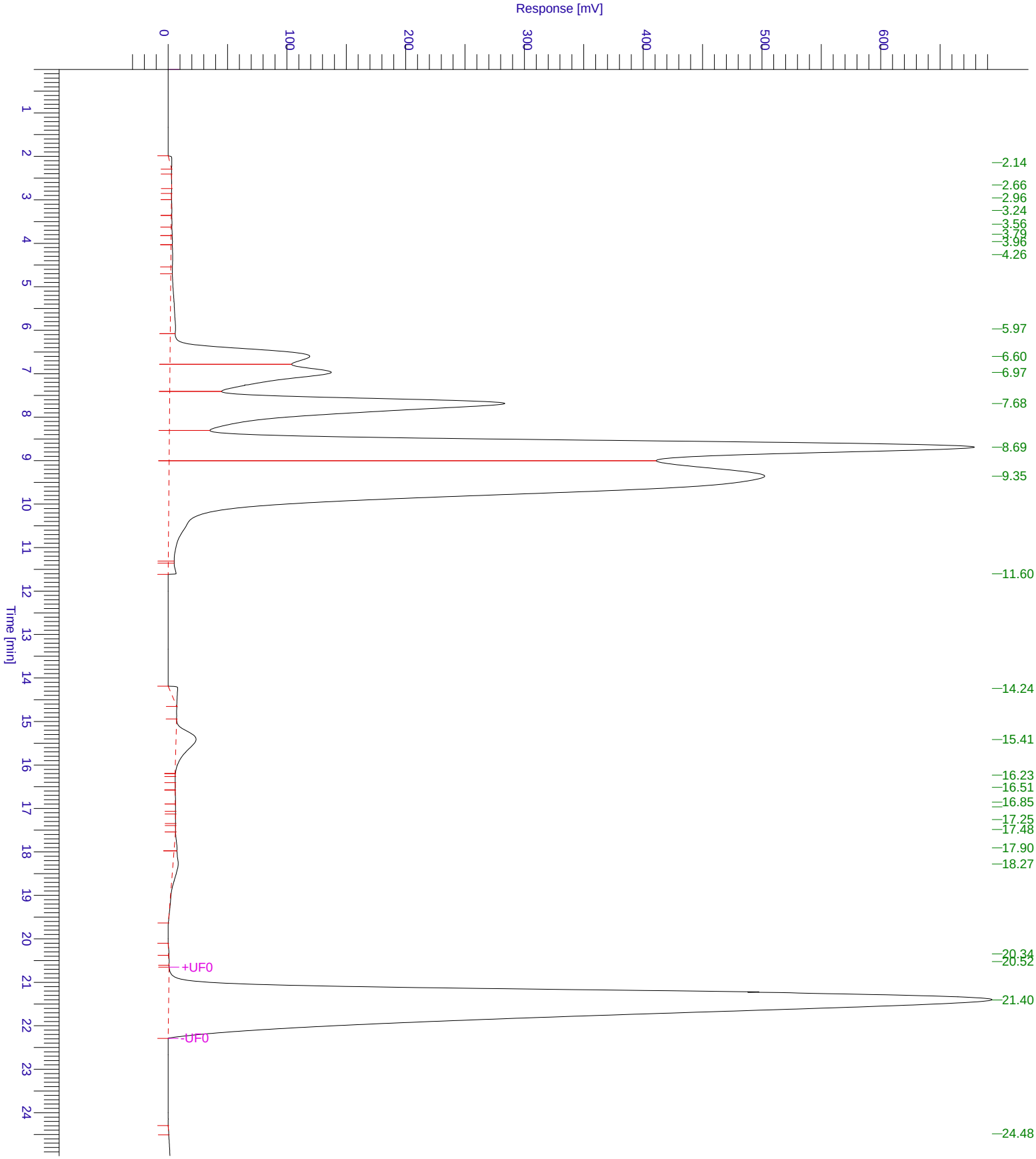
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S72 Sample #: 079 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol079.raw
Date : 6/21/2013 2:18:31 PM
Method : Method Robin 87H Time of Injection: 6/12/2013 11:31:09 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -34.69 mV High Point : 693.82 mV
Scale Factor: 1.0 Plot Offset: -34.69 mV Plot Scale: 728.5 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:19:06 PM
Operator	: rLuong	Sample Name	: S73
Sample Number	: 080	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/80
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/12/2013 11:57:30 PM	Dilution Factor	: 1.00
		Cycle	: 80

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol080.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol080.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol080.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol080.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol080.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	1.881	13874.62	664.53	0.01	0.01	BV	20.8789
2	1.991	3980.94	755.52	0.00	0.00	VV	5.2691
3	2.044	3784.49	822.78	0.00	0.00	VV	4.5997
4	2.172	4632.35	1131.44	0.00	0.00	VB	4.0942
5	5.123	489078.54	5319.01	0.51	0.51	BV	91.9492
6	5.727	208011.86	6491.43	0.22	0.22	VB	32.0441
7	6.581	1877260.56	92774.00	1.96	1.96	BV	20.2348
8	6.969	1913632.28	89618.86	2.00	2.00	VV	21.3530
9	7.686	3829078.68	189585.60	4.00	4.00	VV	20.1971
10	8.087	747036.36	64239.71	0.78	0.78	VV	11.6289
11	8.704	18125589.57	709916.56	18.93	18.93	VV	25.5320
12	9.466	33901589.67	658895.06	35.40	35.40	VE	51.4522
13	11.792	991716.40	11513.95	1.04	1.04	EV	86.1317
14	12.889	244855.91	8638.68	0.26	0.26	VV	28.3441
15	13.473	477054.17	11440.94	0.50	0.50	VB	41.6971
16	14.856	3554.52	608.01	0.00	0.00	BV	5.8461
17	15.423	450994.68	7088.81	0.47	0.47	VV	63.6207
18	16.753	427119.12	14302.58	0.45	0.45	VB	29.8631
19	18.963	479125.00	16036.14	0.50	0.50	BB	29.8778
20	21.413	31493054.91	794695.31	32.89	32.89	MM	39.6291
21	23.548	33787.19	1569.29	0.04	0.04	BV	21.5302
22	23.858	44407.61	1576.39	0.05	0.05	VB	28.1705
		95763219.43	2.69e+06	100.00	100.00		

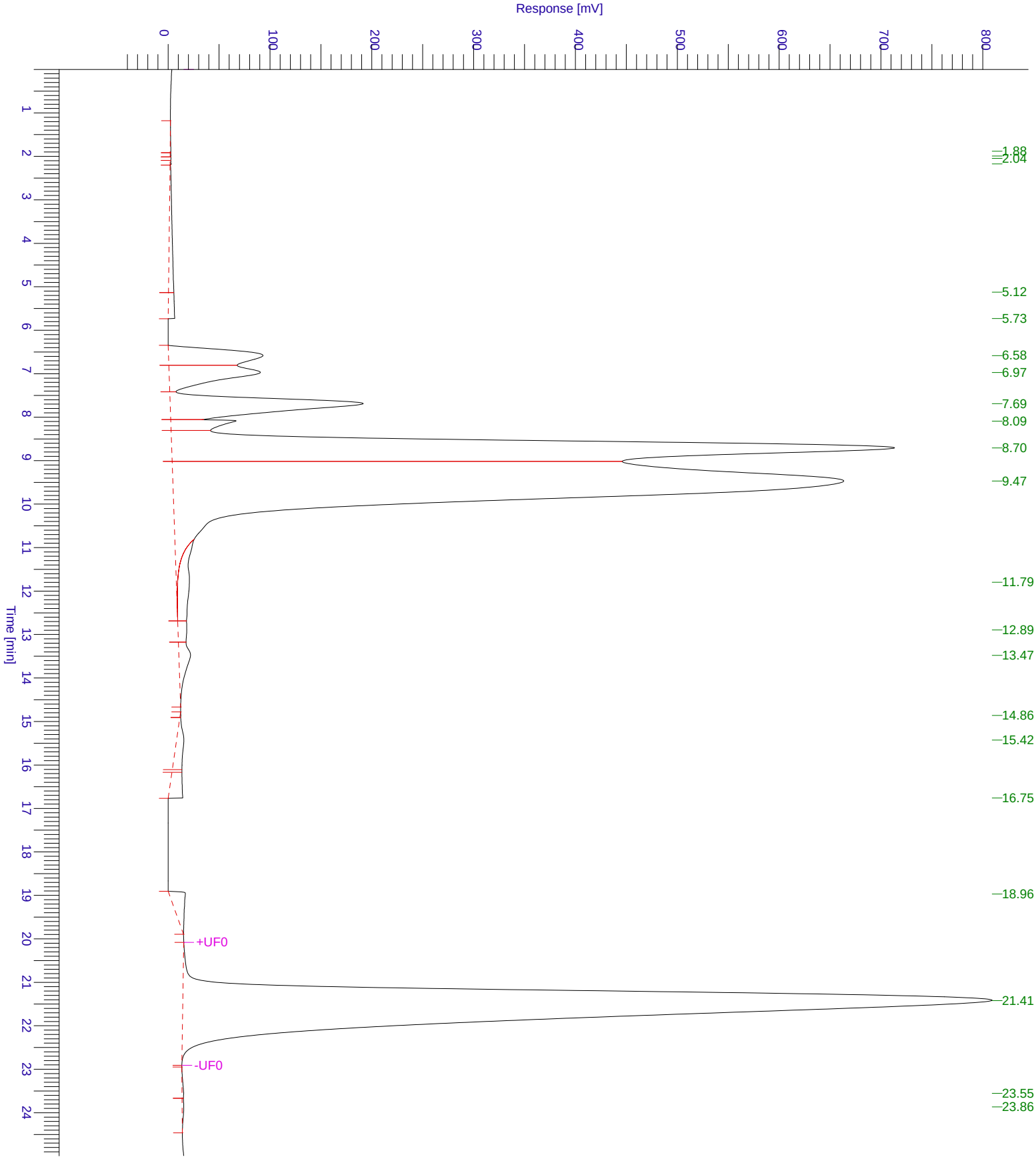
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S73 Sample #: 080 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol080.raw
Date : 6/21/2013 2:19:31 PM
Method : Method Robin 87H Time of Injection: 6/12/2013 11:57:30 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -40.46 mV High Point : 809.16 mV
Scale Factor: 1.0 Plot Offset: -40.46 mV Plot Scale: 849.6 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:20:09 PM
Operator	: rLuong	Sample Name	: S74
Sample Number	: 081	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/81
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/13/2013 12:23:49 AM	Dilution Factor	: 1.00
		Cycle	: 81

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol081.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol081.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol081.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol081.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol081.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	1.789	107649.01	11554.55	0.11	0.11	BV	9.3166
2	1.901	192068.08	11435.28	0.20	0.20	VV	16.7961
3	3.879	868829.85	6880.47	0.91	0.91	VV	126.2748
4	4.168	300968.32	6147.83	0.32	0.32	VB	48.9552
5	5.713	1074.80	236.04	0.00	0.00	BB	4.5535
6	6.587	2786875.17	125418.83	2.93	2.93	BV	22.2205
7	6.969	3234714.60	126113.75	3.40	3.40	VV	25.6492
8	7.687	6053751.31	233852.64	6.37	6.37	VV	25.8870
9	8.698	17975934.72	711028.25	18.91	18.91	VV	25.2816
10	9.473	31760119.79	593459.86	33.42	33.42	VB	53.5169
11	14.027	86429.00	7077.49	0.09	0.09	BB	12.2118
12	15.419	180107.40	5458.19	0.19	0.19	BB	32.9977
13	16.830	378.40	75.29	0.00	0.00	BB	5.0256
14	17.061	428.20	74.44	0.00	0.00	BB	5.7525
15	18.401	154765.00	4301.92	0.16	0.16	BB	35.9758
16	19.913	342.40	64.50	0.00	0.00	BB	5.3085
17	21.400	31339491.77	796411.21	32.97	32.97	MM	39.3509
		95043927.82	2.64e+06	100.00	100.00		

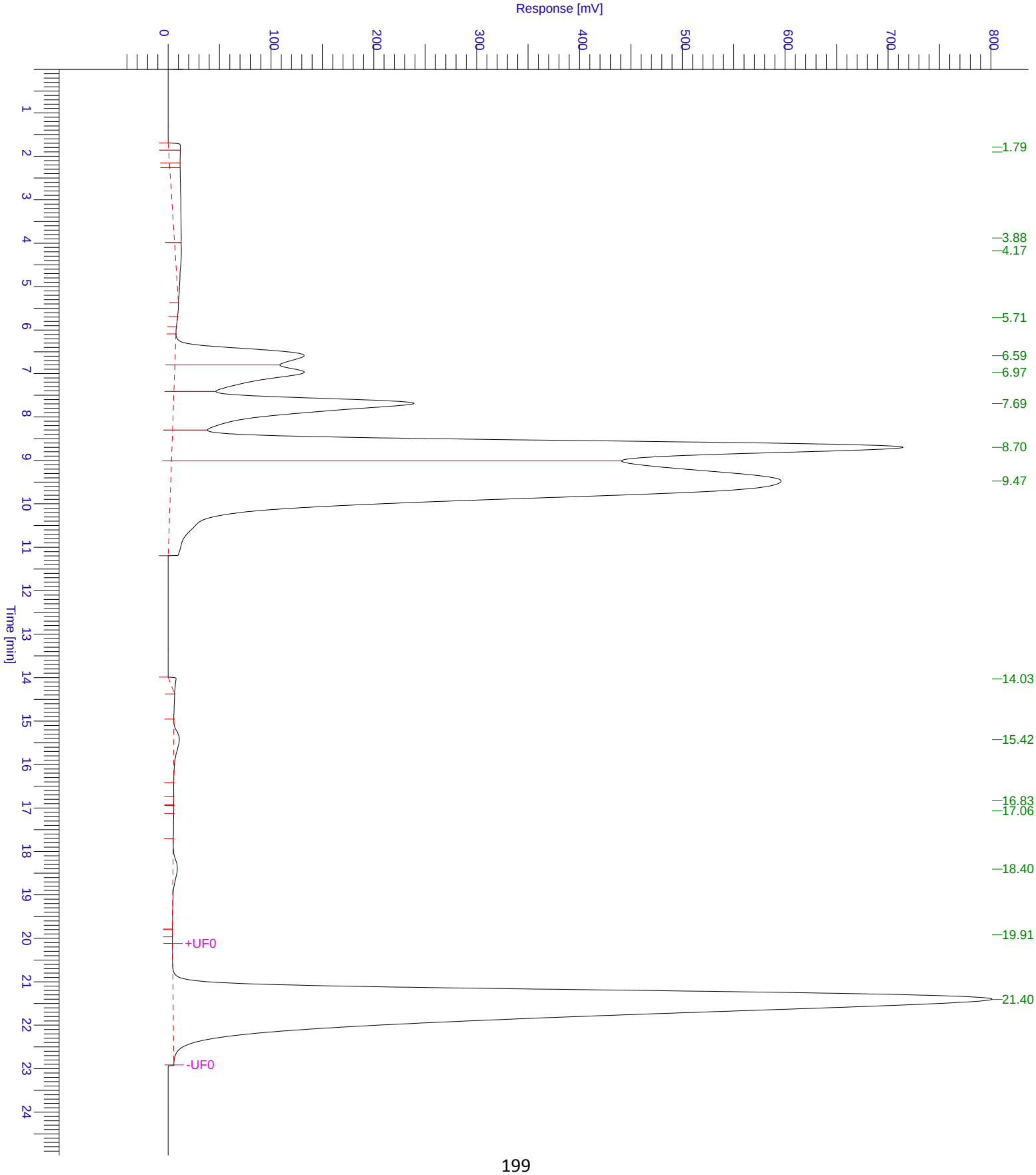
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S74 Sample #: 081 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol081.raw
Date : 6/21/2013 2:20:35 PM
Method : Method Robin 87H Time of Injection: 6/13/2013 12:23:49 AM
Start Time : 0.00 min End Time : 24.99 min Low Point : -40.06 mV High Point : 801.23 mV
Scale Factor: 1.0 Plot Offset: -40.06 mV Plot Scale: 841.3 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:21:14 PM
Operator	: rLuong	Sample Name	: S75
Sample Number	: 082	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/82
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/13/2013 12:50:09 AM	Dilution Factor	: 1.00
		Cycle	: 82

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol082.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol082.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol082.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol082.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol082.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.039	250.40	125.11	0.00	0.00	BB	2.0015
2	0.181	1418.94	310.91	0.00	0.00	BV	4.5638
3	0.372	3818.06	635.19	0.00	0.00	VB	6.0109
4	0.444	1757.53	680.31	0.00	0.00	BV	2.5834
5	0.538	4267.29	588.33	0.00	0.00	VV	7.2533
6	1.033	22682.64	703.74	0.02	0.02	VV	32.2314
7	1.316	997.74	115.48	0.00	0.00	VB	8.6400
8	5.068	116858.22	3474.26	0.12	0.12	BV	33.6354
9	5.588	130948.00	5273.23	0.14	0.14	VV	24.8326
10	5.926	116977.50	6463.56	0.12	0.12	VV	18.0980
11	6.147	91363.22	8570.20	0.10	0.10	VB	10.6606
12	6.579	1960632.72	95579.19	2.05	2.05	BV	20.5132
13	6.966	2036378.01	93460.10	2.13	2.13	VV	21.7887
14	7.684	4267375.41	196232.48	4.47	4.47	VV	21.7465
15	8.702	17475333.98	717628.93	18.29	18.29	VV	24.3515
16	9.475	32688798.88	599004.65	34.20	34.20	VE	54.5719
17	11.620	951162.40	10570.36	1.00	1.00	EV	89.9839
18	13.659	1372101.18	15725.60	1.44	1.44	VV	87.2527
19	15.425	2625097.86	24271.28	2.75	2.75	VV	108.1565
20	17.660	45260.96	7558.00	0.05	0.05	VB	5.9885
21	21.407	31658412.46	789438.46	33.13	33.13	MM	40.1024
		95571893.40	2.58e+06	100.00	100.00		

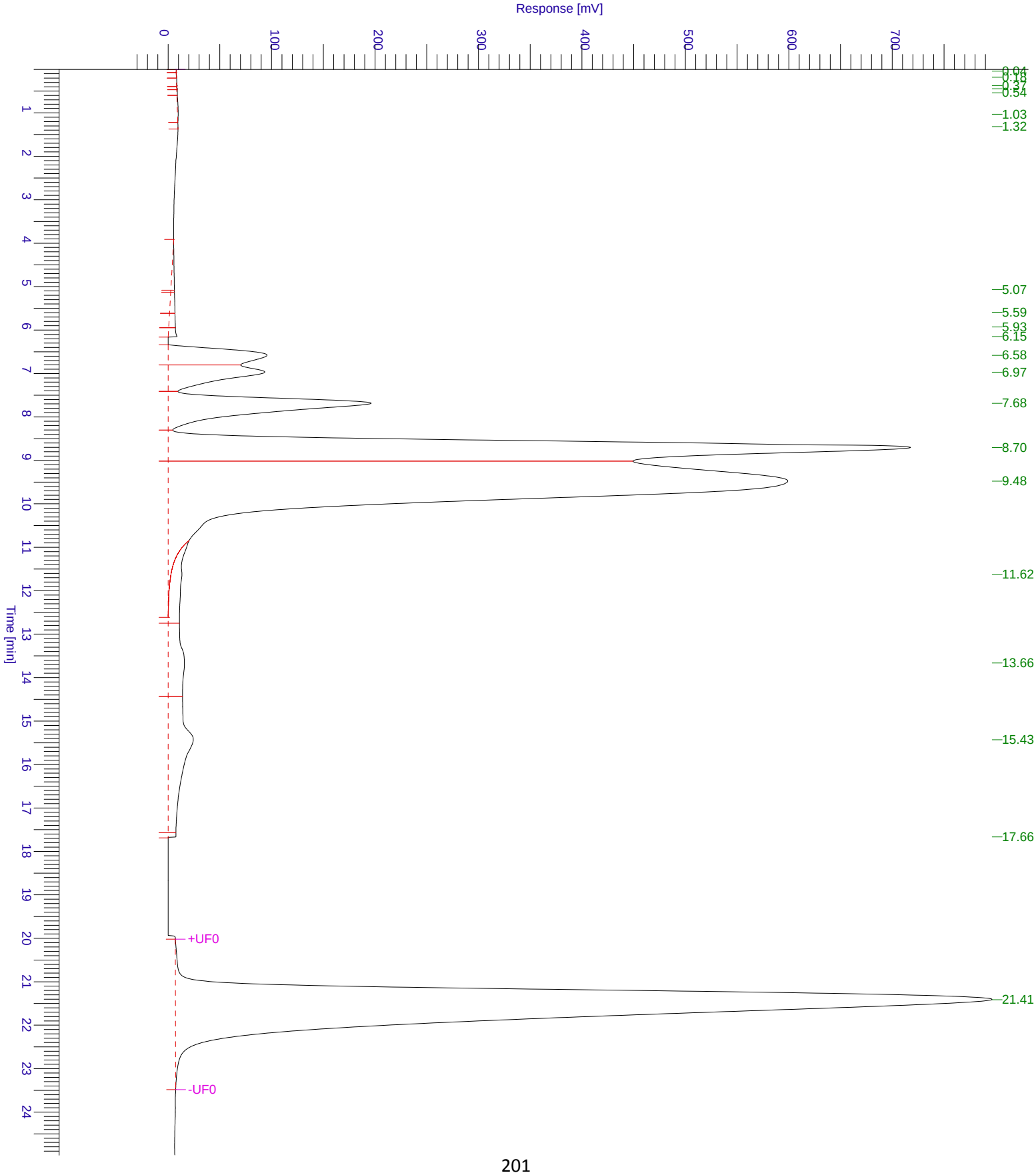
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S75 Sample #: 082 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol082.raw
Date : 6/21/2013 2:21:36 PM
Method : Method Robin 87H Time of Injection: 6/13/2013 12:50:09 AM
Start Time : 0.00 min End Time : 24.99 min Low Point : -39.82 mV High Point : 796.50 mV
Scale Factor: 1.0 Plot Offset: -39.82 mV Plot Scale: 836.3 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:22:46 PM
Operator	: rLuong	Sample Name	: S76
Sample Number	: 083	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/83
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/13/2013 1:16:29 AM	Dilution Factor	: 1.00
		Cycle	: 83

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol083.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol083.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol083.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol083.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol083.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

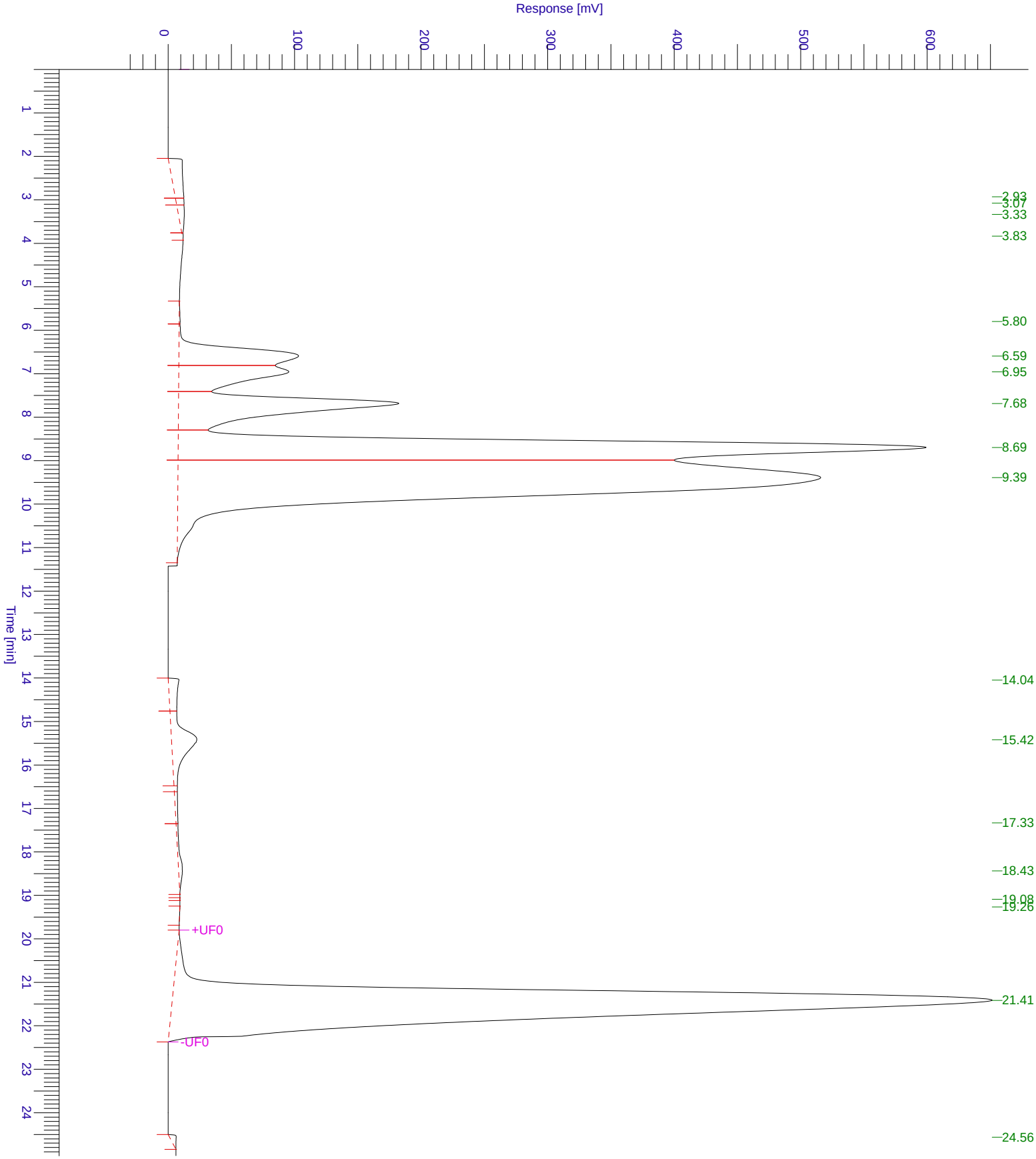
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	2.929	472751.04	6849.23	0.62	0.62	BV	69.0225
2	3.074	59059.94	6140.59	0.08	0.08	VV	9.6180
3	3.333	143983.31	4664.00	0.19	0.19	VV	30.8712
4	3.832	5723.91	671.58	0.01	0.01	VB	8.5230
5	5.798	12879.06	733.22	0.02	0.02	BV	17.5650
6	6.591	2229977.25	94529.94	2.90	2.90	VV	23.5902
7	6.954	2165560.87	86942.08	2.82	2.82	VV	24.9081
8	7.683	4429474.41	174159.35	5.76	5.76	VV	25.4335
9	8.695	14659402.38	591216.19	19.08	19.08	VV	24.7953
10	9.387	25331466.03	508035.62	32.97	32.97	VB	49.8616
11	14.044	298708.01	8441.92	0.39	0.39	BV	35.3839
12	15.415	922505.14	20032.17	1.20	1.20	VV	46.0512
13	17.327	84759.80	1552.32	0.11	0.11	VV	54.6021
14	18.430	160170.79	2978.53	0.21	0.21	VB	53.7751
15	19.083	86.20	44.47	0.00	0.00	BB	1.9382
16	19.259	1279.00	70.83	0.00	0.00	BB	18.0572
17	21.410	25798750.81	648138.45	33.58	33.58	MM	39.8044
18	24.556	61414.00	5310.61	0.08	0.08	BB	11.5644
		76837951.96	2.16e+06	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S76 Sample #: 083 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol083.raw
Date : 6/21/2013 2:23:09 PM
Method : Method Robin 87H Time of Injection: 6/13/2013 1:16:29 AM
Start Time : 0.00 min End Time : 24.99 min Low Point : -32.56 mV High Point : 651.32 mV
Scale Factor: 1.0 Plot Offset: -32.56 mV Plot Scale: 683.9 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 2:24:13 PM
Operator	: rLuong	Sample Name	: DDW1
Sample Number	: 084	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/1
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 500.11 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/13/2013 1:41:42 AM	Dilution Factor	: 1.00
		Cycle	: 84

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol084-20130613-032138.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol084.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin Low Flow 87P from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol084-20130613-032138.raw
Proc Method : C:\HPLC Data\robin\Method Robin Low Flow 87P from c:\hplc data\robin\ethanol_june 10-2013\ethanol084.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin Low Flow 87P from c:\hplc data\robin\ethanol_june 10-2013\ethanol084.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin Low Flow 87P.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	1.737	598383.67	3683.49	0.89	0.89	BV	162.4500
2	8.371	2181450.33	9106.00	3.26	3.26	VV	239.5618
3	15.067	1729283.60	9651.46	2.58	2.58	VB	179.1732
4	24.560	513771.09	14507.60	0.77	0.77	BV	35.4139
5	28.800	3450459.91	11283.30	5.15	5.15	VV	305.8024
6	35.630	2752912.91	7701.94	4.11	4.11	VB	357.4310
7	49.367	438852.00	8231.40	0.65	0.65	BB	53.3144
8	54.217	1094832.00	7634.97	1.63	1.63	BB	143.3970
9	74.853	1356032.80	11826.54	2.02	2.02	BB	114.6601
10	91.320	1058878.80	2999.25	1.58	1.58	BB	353.0477
11	95.891	1055982.10	8268.91	1.58	1.58	BV	127.7051
12	98.805	3635594.99	14951.25	5.43	5.43	VV	243.1633
13	103.593	4165483.30	24268.42	6.22	6.22	VB	171.6421
14	116.769	2149730.00	13103.41	3.21	3.21	BB	164.0588
15	122.533	982909.00	18212.32	1.47	1.47	BB	53.9695
16	135.180	556382.40	14922.90	0.83	0.83	BB	37.2838
17	141.493	118848.40	1233.73	0.18	0.18	BB	96.3323
18	160.973	2562830.83	11232.37	3.82	3.82	BV	228.1647
19	164.767	651247.97	2257.09	0.97	0.97	VB	288.5342
20	178.867	808412.00	7519.79	1.21	1.21	BB	107.5046
21	198.580	3183128.00	3220.05	4.75	4.75	BB	988.5336
22	209.653	19740348.00	94348.00	29.46	29.46	BB	209.2291
23	212.127	626527.20	4426.26	0.94	0.94	BB	141.5477
24	231.793	308673.80	7966.20	0.46	0.46	BB	38.7479
25	251.413	741608.40	5472.60	1.11	1.11	BB	135.5129
26	270.567	900839.60	5101.14	1.34	1.34	BB	176.5956
27	288.813	277665.60	7571.82	0.41	0.41	BB	36.6709
28	304.151	490448.40	9356.95	0.73	0.73	BB	52.4154
29	324.907	798483.20	976.32	1.19	1.19	BB	817.8504
30	337.067	285767.60	5021.44	0.43	0.43	BB	56.9094
31	358.820	1159072.00	798.75	1.73	1.73	BB	1451.1101
32	376.387	1206408.40	737.11	1.80	1.80	BB	1636.6798
33	388.560	127715.60	4637.69	0.19	0.19	BB	27.5386
34	391.433	24790.40	489.55	0.04	0.04	BB	50.6390
35	408.220	1152236.20	4082.84	1.72	1.72	BB	282.2141
36	432.526	923327.00	4816.08	1.38	1.38	BB	191.7177
37	444.593	303966.40	6294.50	0.45	0.45	BB	48.2908
38	454.967	1293316.80	8435.90	1.93	1.93	BB	153.3111
39	469.633	1361289.20	3421.97	2.03	2.03	BB	397.8082
40	485.973	237178.40	3977.84	0.35	0.35	BB	59.6250
		67005068.31	383749.18	100.00	100.00		

6/21/2013 2:24:13 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol084.rst

Warning -- Signal level out-of-range in peak

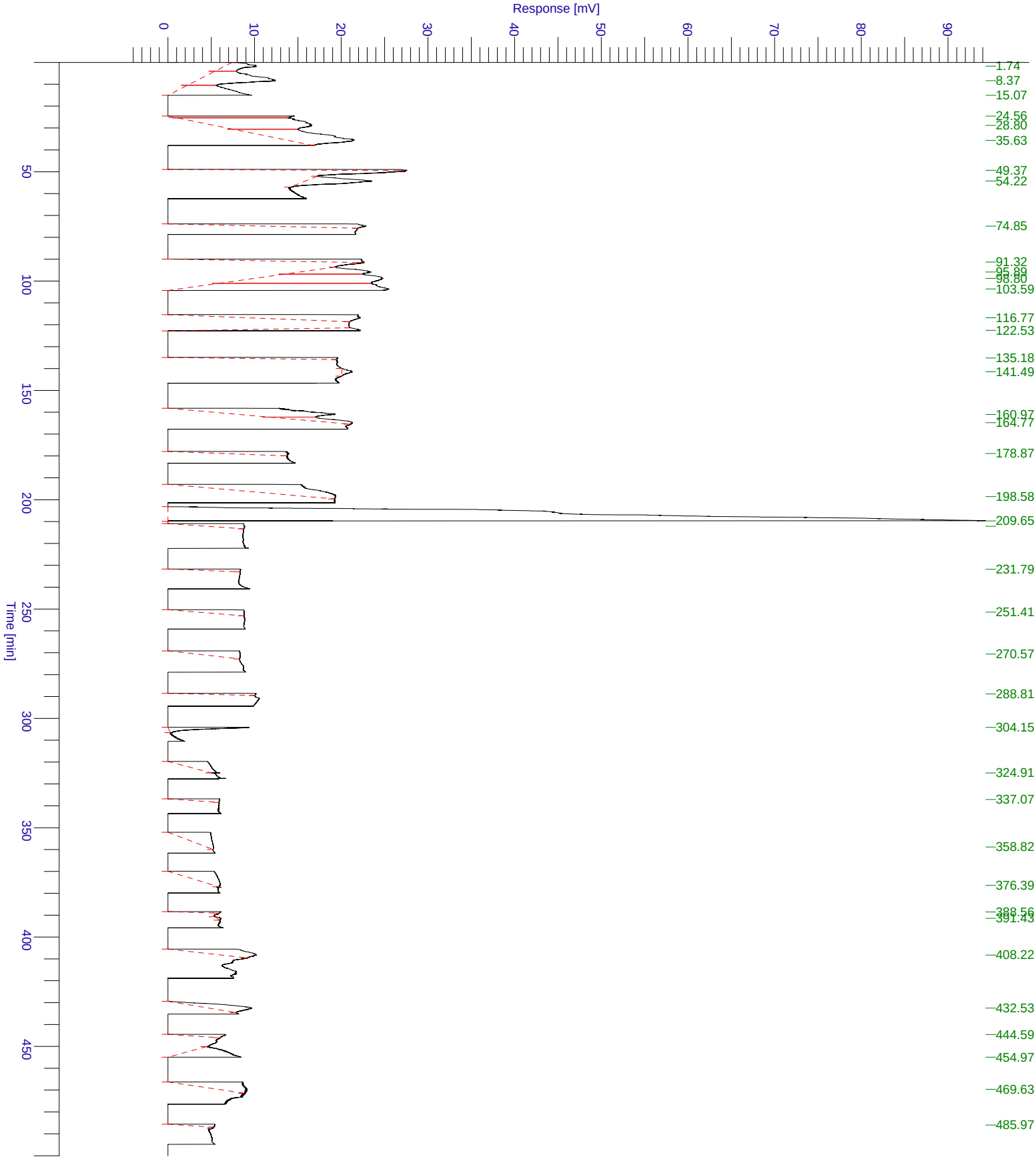
Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Chromatogram

Sample Name : DDW1 Sample #: 084 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol084-20130613-032138.raw
Date : 6/21/2013 2:24:51 PM
Method : Method Robin Low Flow 87P Time of Injection: 6/13/2013 1:41:42 AM
Start Time : 0.00 min End Time : 500.11 min Low Point : -4.72 mV High Point : 94.35 mV
Scale Factor: 1.0 Plot Offset: -4.72 mV Plot Scale: 99.1 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:56:22 PM
Operator	: rLuong	Sample Name	: S8
Sample Number	: 015	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/15
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 7:27:41 PM	Dilution Factor	: 1.00
		Cycle	: 15

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol015.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol015.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol015.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol015.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol015.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.110	131.60	59.19	0.00	0.00	BB	2.2234
2	0.263	120.00	79.21	0.00	0.00	BB	1.5150
3	0.362	87.20	48.60	8e-05	8e-05	BB	1.7942
4	0.474	69.46	72.15	6e-05	6e-05	BV	0.9626
5	0.501	120.54	87.98	0.00	0.00	VB	1.3701
6	0.735	342.60	91.07	0.00	0.00	BB	3.7621
7	0.941	369.60	121.34	0.00	0.00	BB	3.0460
8	1.162	67.00	65.09	6e-05	6e-05	BB	1.0293
9	1.237	99.60	49.87	9e-05	9e-05	BB	1.9972
10	1.393	580.60	148.88	0.00	0.00	BB	3.8998
11	1.539	83.00	60.47	7e-05	7e-05	BB	1.3726
12	1.649	220.00	73.63	0.00	0.00	BB	2.9880
13	1.919	167.20	93.53	0.00	0.00	BB	1.7877
14	2.026	358.80	86.64	0.00	0.00	BB	4.1412
15	2.201	524.00	98.94	0.00	0.00	BV	5.2959
16	2.342	352.00	78.27	0.00	0.00	VB	4.4975
17	2.660	85.00	53.91	8e-05	8e-05	BB	1.5768
18	2.987	264.00	52.88	0.00	0.00	BB	4.9922
19	3.153	113.80	57.08	0.00	0.00	BB	1.9938
20	3.566	131.20	57.62	0.00	0.00	BB	2.2771
21	4.431	246.40	66.27	0.00	0.00	BB	3.7183
22	5.582	61.60	51.31	5e-05	5e-05	BB	1.2006
23	5.729	129.60	58.24	0.00	0.00	BB	2.2254
24	5.882	272.20	111.92	0.00	0.00	BB	2.4321
25	6.566	2988995.59	108708.12	2.67	2.67	BV	27.4956
26	7.301	3205027.29	86463.83	2.86	2.86	VV	37.0678
27	8.605	16994992.43	693895.75	15.16	15.16	VV	24.4921
28	9.267	55802536.10	985875.96	49.79	49.79	VB	56.6020
29	12.771	280.40	112.76	0.00	0.00	BB	2.4867
30	13.384	3482.00	173.43	0.00	0.00	BB	20.0775
31	14.088	386.40	133.70	0.00	0.00	BB	2.8901
32	14.386	613.40	135.80	0.00	0.00	BB	4.5170
33	14.676	102.03	124.79	9e-05	9e-05	BV	0.8176
34	14.701	395.97	126.28	0.00	0.00	VB	3.1357
35	14.927	888.78	157.81	0.00	0.00	BV	5.6319
36	14.961	310.20	201.43	0.00	0.00	VV	1.5400
37	15.380	134317.90	4529.43	0.12	0.12	VV	29.6545
38	15.860	6289.92	888.98	0.01	0.01	VB	7.0755
39	16.152	501.48	210.06	0.00	0.00	BV	2.3874
40	16.220	103.92	53.81	9e-05	9e-05	VB	1.9311
41	16.436	131.80	82.88	0.00	0.00	BB	1.5903
42	16.706	311.60	126.33	0.00	0.00	BB	2.4666
43	16.922	72.40	58.69	6e-05	6e-05	BB	1.2336
44	16.984	344.80	119.99	0.00	0.00	BB	2.8736
45	17.444	196.89	56.19	0.00	0.00	BV	3.5037

6/21/2013 12:56:22 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol015.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	18.314	102413.11	2532.85	0.09	0.09	VB	40.4340
47	19.217	214.40	91.34	0.00	0.00	BB	2.3472
48	19.465	132.00	67.68	0.00	0.00	BB	1.9504
49	20.261	812.12	158.44	0.00	0.00	BV	5.1257
50	20.527	4733.13	310.38	0.00	0.00	VV	15.2493
51	21.518	32820006.20	819878.16	29.28	29.28	VB	40.0303
52	24.169	174.80	64.44	0.00	0.00	BB	2.7127
53	24.342	375.20	69.43	0.00	0.00	BB	5.4043
54	24.819	86.80	35.13	8e-05	8e-05	BB	2.4711
55	24.938	192.20	82.38	0.00	0.00	BB	2.3331
		1.12e+08	2.71e+06	100.00	100.00		

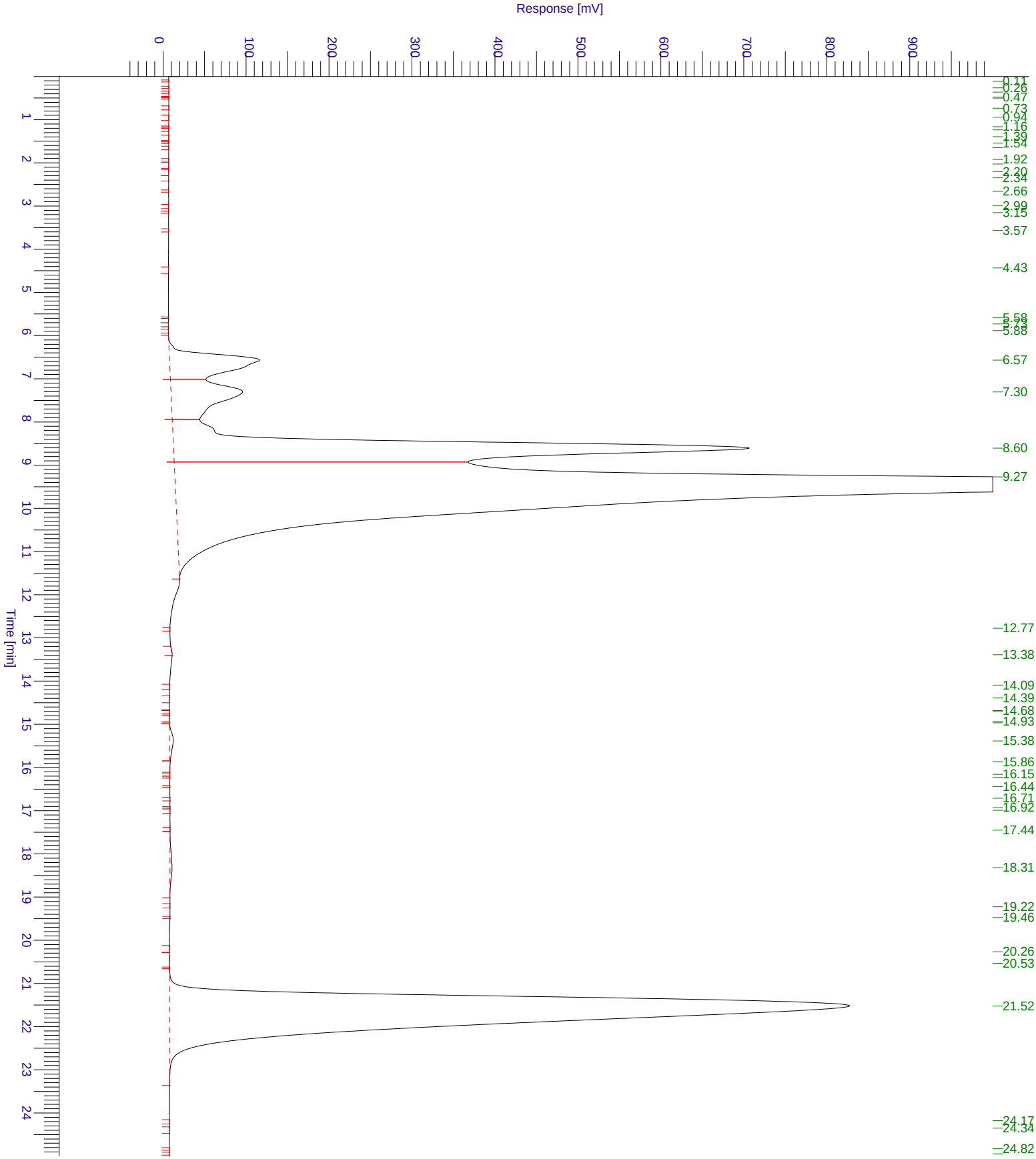
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S8 Sample #: 015 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol015.raw
Date : 6/21/2013 12:56:41 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 7:27:41 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -43.48 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -43.48 mV Plot Scale: 1043.5 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:57:06 PM
Operator	: rLuong	Sample Name	: S9
Sample Number	: 016	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/16
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 7:53:57 PM	Dilution Factor	: 1.00
		Cycle	: 16

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol016.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol016.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol016.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol016.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol016.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.186	154.80	53.80	0.00	0.00	BB	2.8774
2	0.245	226.40	89.26	0.00	0.00	BB	2.5366
3	0.321	354.40	131.00	0.00	0.00	BB	2.7053
4	0.517	146.41	88.26	0.00	0.00	BV	1.6589
5	0.569	132.39	76.65	0.00	0.00	VB	1.7271
6	0.813	788.40	175.08	0.00	0.00	BB	4.5032
7	1.096	891.00	116.84	0.00	0.00	BB	7.6260
8	1.256	124.91	66.03	0.00	0.00	BV	1.8918
9	1.315	171.59	109.06	0.00	0.00	VB	1.5733
10	1.372	164.20	81.44	0.00	0.00	BB	2.0161
11	1.562	100.61	95.76	9e-05	9e-05	BV	1.0507
12	1.595	138.99	96.92	0.00	0.00	VB	1.4340
13	1.759	94.19	93.85	8e-05	8e-05	BV	1.0036
14	1.788	131.41	88.85	0.00	0.00	VB	1.4790
15	1.817	54.00	75.86	5e-05	5e-05	BB	0.7119
16	1.850	276.00	128.83	0.00	0.00	BB	2.1424
17	1.944	163.80	44.20	0.00	0.00	BB	3.7056
18	2.207	139.60	87.59	0.00	0.00	BB	1.5938
19	2.439	584.35	114.93	0.00	0.00	BV	5.0843
20	2.528	127.25	63.85	0.00	0.00	VB	1.9931
21	2.835	126.82	62.41	0.00	0.00	BV	2.0321
22	2.913	211.93	75.31	0.00	0.00	VV	2.8140
23	2.939	112.85	103.79	0.00	0.00	VB	1.0873
24	2.965	107.00	107.56	1e-04	1e-04	BB	0.9948
25	3.005	290.00	154.11	0.00	0.00	BB	1.8818
26	3.169	210.58	89.03	0.00	0.00	BV	2.3651
27	3.220	149.42	68.02	0.00	0.00	VB	2.1969
28	3.440	172.80	92.45	0.00	0.00	BB	1.8691
29	3.490	101.06	81.65	9e-05	9e-05	BV	1.2378
30	3.528	428.34	103.90	0.00	0.00	VB	4.1224
31	3.609	87.77	84.48	8e-05	8e-05	BV	1.0390
32	3.645	291.78	142.41	0.00	0.00	VV	2.0489
33	3.693	381.85	109.21	0.00	0.00	VB	3.4963
34	4.181	542.80	178.71	0.00	0.00	BB	3.0374
35	4.294	56.80	59.12	5e-05	5e-05	BB	0.9607
36	4.332	203.60	96.91	0.00	0.00	BB	2.1008
37	4.542	328.40	117.90	0.00	0.00	BB	2.7853
38	4.731	698.00	90.27	0.00	0.00	BB	7.7325
39	4.961	181.20	75.93	0.00	0.00	BB	2.3865
40	5.018	728.00	218.82	0.00	0.00	BB	3.3269
41	5.285	182.80	84.34	0.00	0.00	BB	2.1675
42	5.475	494.40	109.92	0.00	0.00	BB	4.4977
43	5.701	96.80	76.09	9e-05	9e-05	BB	1.2722
44	5.732	53.20	55.33	5e-05	5e-05	BB	0.9615
45	5.795	221.60	71.51	0.00	0.00	BB	3.0990

6/21/2013 12:57:06 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol016.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	6.014	566.12	194.74	0.00	0.00	BV	2.9071
47	6.570	3062468.13	108498.92	2.74	2.74	VV	28.2258
48	7.302	3352790.61	89893.32	3.00	3.00	VV	37.2974
49	8.614	17473367.32	693450.42	15.64	15.64	VV	25.1977
50	9.280	55286945.02	997493.21	49.47	49.47	VB	55.4259
51	16.666	15013.20	2253.60	0.01	0.01	BB	6.6619
52	16.870	274.00	72.69	0.00	0.00	BB	3.7696
53	16.942	682.59	199.08	0.00	0.00	BV	3.4288
54	17.040	169.75	63.66	0.00	0.00	VV	2.6667
55	17.087	2140.93	336.68	0.00	0.00	VV	6.3589
56	17.234	634.06	214.93	0.00	0.00	VV	2.9501
57	17.287	93.35	88.34	8e-05	8e-05	VV	1.0568
58	17.353	686.72	140.71	0.00	0.00	VB	4.8805
59	17.537	442.15	127.98	0.00	0.00	BV	3.4549
60	17.594	139.05	94.32	0.00	0.00	VB	1.4744
61	17.639	232.22	129.60	0.00	0.00	BV	1.7918
62	17.681	608.66	169.84	0.00	0.00	VV	3.5837
63	18.273	35618.89	1965.37	0.03	0.03	VV	18.1232
64	18.307	36891.95	2020.01	0.03	0.03	VV	18.2633
65	18.770	804.84	437.36	0.00	0.00	VV	1.8402
66	18.791	1634.63	419.62	0.00	0.00	VB	3.8955
67	18.915	101.80	71.50	9e-05	9e-05	BV	1.4238
68	18.957	101.00	76.29	9e-05	9e-05	VB	1.3239
69	18.999	181.20	130.43	0.00	0.00	BV	1.3892
70	19.048	474.80	169.47	0.00	0.00	VB	2.8016
71	19.240	1139.25	193.78	0.00	0.00	BV	5.8791
72	19.303	382.35	81.39	0.00	0.00	VB	4.6976
73	19.676	100.99	133.74	9e-05	9e-05	BV	0.7552
74	19.702	1384.41	179.24	0.00	0.00	VB	7.7239
75	20.046	43.80	46.54	4e-05	4e-05	BB	0.9412
76	20.167	328.18	93.95	0.00	0.00	BV	3.4931
77	20.246	299.04	101.67	0.00	0.00	VV	2.9412
78	20.311	57.38	71.70	5e-05	5e-05	VB	0.8004
79	20.345	523.04	131.46	0.00	0.00	BV	3.9786
80	20.449	421.31	152.71	0.00	0.00	VV	2.7590
81	20.571	321.85	98.87	0.00	0.00	VB	3.2555
82	20.604	98.00	73.31	9e-05	9e-05	BB	1.3367
83	20.781	1923.65	405.76	0.00	0.00	BV	4.7409
84	21.529	32456699.35	810278.62	29.04	29.04	VB	40.0562
85	23.451	390.00	141.40	0.00	0.00	BB	2.7582
86	23.706	68.20	71.41	6e-05	6e-05	BB	0.9551
87	23.742	243.20	98.11	0.00	0.00	BB	2.4787
88	24.022	272.80	94.37	0.00	0.00	BB	2.8908
89	24.168	373.60	56.62	0.00	0.00	BB	6.5985
90	24.287	60.60	62.39	5e-05	5e-05	BB	0.9713
91	24.327	156.00	61.68	0.00	0.00	BB	2.5290
92	24.586	65.80	66.27	6e-05	6e-05	BB	0.9929
93	24.619	107.00	68.05	1e-04	1e-04	BB	1.5723
94	24.742	188.80	54.67	0.00	0.00	BB	3.4536
95	24.889	69.08	65.11	6e-05	6e-05	BV	1.0609
96	24.924	174.12	113.84	0.00	0.00	VB	1.5295
		1.12e+08	2.72e+06	100.00	100.00		

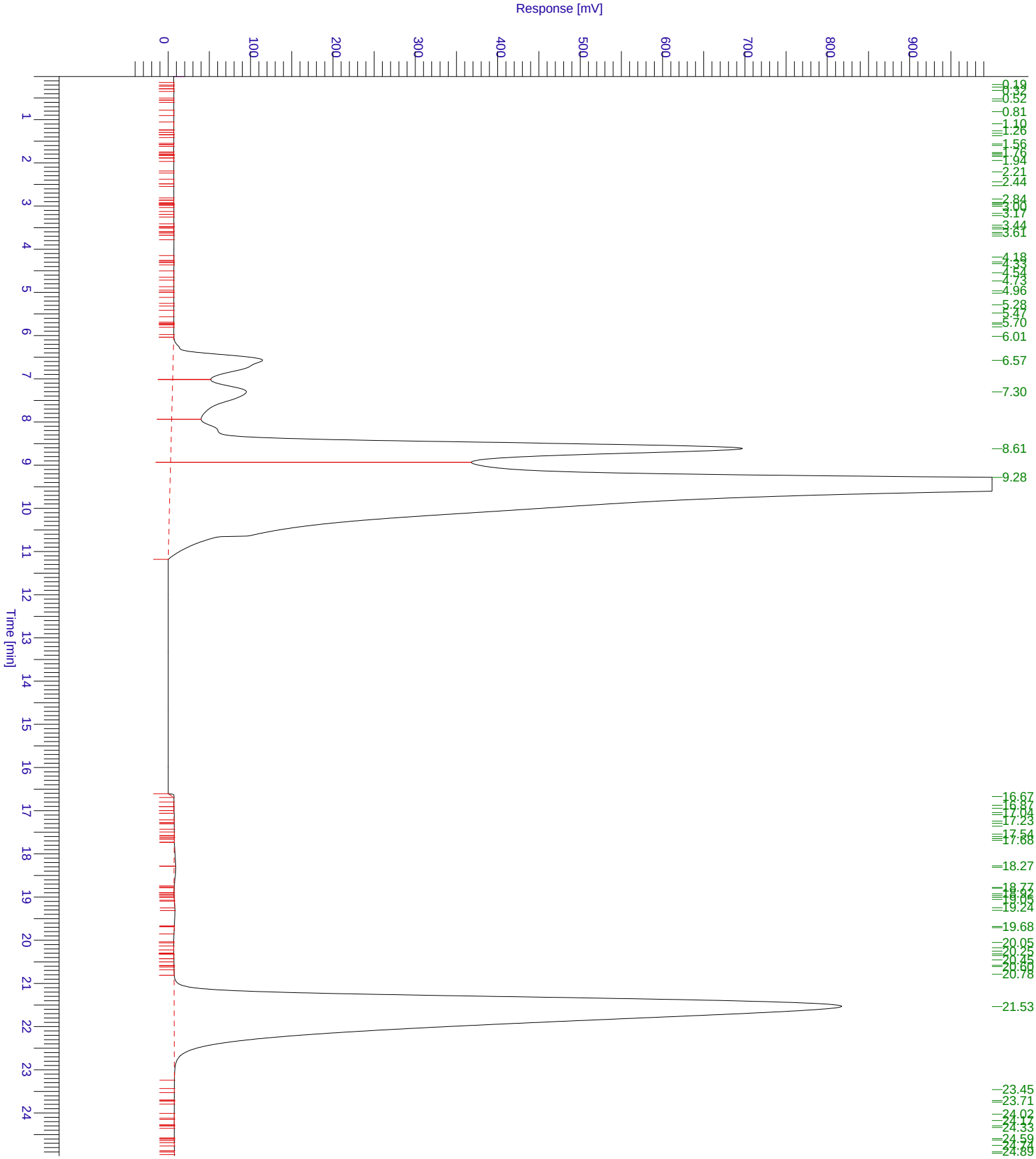
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : S9 Sample #: 016 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol016.raw
Date : 6/21/2013 12:57:29 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 7:53:57 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -50.00 mV High Point : 1000.00 mV
Scale Factor: 1.0 Plot Offset: -50.00 mV Plot Scale: 1050.0 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:41:08 PM
Operator	: rLuong	Sample Name	: St1
Sample Number	: 003	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/3
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 2:12:29 PM	Dilution Factor	: 1.00
		Cycle	: 3

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol003.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol003.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol003.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol003.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol003.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.096	105.40	77.96	0.02	0.02	BB	1.3521
2	0.290	763.20	96.66	0.13	0.13	BB	7.8955
3	1.398	283.20	63.58	0.05	0.05	BB	4.4540
4	1.556	373.80	86.96	0.06	0.06	BB	4.2985
5	1.713	253.80	66.50	0.04	0.04	BB	3.8167
6	2.021	173.80	49.99	0.03	0.03	BB	3.4765
7	2.838	394.00	59.28	0.07	0.07	BB	6.6464
8	3.008	94.40	37.10	0.02	0.02	BB	2.5445
9	3.479	61.40	28.03	0.01	0.01	BB	2.1904
10	3.658	157.20	47.50	0.03	0.03	BB	3.3097
11	4.117	320.60	67.88	0.06	0.06	BB	4.7228
12	4.301	343.11	75.21	0.06	0.06	BV	4.5623
13	4.454	225.29	46.21	0.04	0.04	VB	4.8754
14	4.632	153.60	44.34	0.03	0.03	BB	3.4645
15	5.246	12751.90	1099.48	2.21	2.21	BV	11.5981
16	5.929	118719.26	4397.66	20.58	20.58	VV	26.9960
17	6.100	60590.78	5202.63	10.50	10.50	VV	11.6462
18	6.207	49666.05	5721.05	8.61	8.61	VB	8.6813
19	7.707	149512.86	2656.09	25.92	25.92	BV	56.2907
20	7.835	16372.79	2018.68	2.84	2.84	VV	8.1106
21	7.985	11592.86	1205.69	2.01	2.01	VV	9.6151
22	8.129	2052.29	369.41	0.36	0.36	VB	5.5555
23	8.311	249.00	58.07	0.04	0.04	BB	4.2876
24	9.097	16011.20	780.96	2.78	2.78	BB	20.5018
25	10.223	100.40	43.85	0.02	0.02	BB	2.2895
26	10.697	145.20	40.34	0.03	0.03	BB	3.5995
27	11.019	115.20	48.69	0.02	0.02	BB	2.3659
28	11.551	1659.60	98.34	0.29	0.29	BB	16.8754
29	12.365	144.40	60.46	0.03	0.03	BB	2.3884
30	12.683	295.40	77.44	0.05	0.05	BB	3.8144
31	12.956	249.20	53.78	0.04	0.04	BB	4.6338
32	13.122	287.20	88.48	0.05	0.05	BB	3.2459
33	13.448	213.00	63.27	0.04	0.04	BB	3.3665
34	13.931	490.97	83.90	0.09	0.09	BV	5.8521
35	14.091	280.23	62.98	0.05	0.05	VB	4.4496
36	14.569	162.80	56.85	0.03	0.03	BB	2.8635
37	15.043	204.80	61.99	0.04	0.04	BB	3.3039
38	15.764	1162.45	41.57	0.20	0.20	BV	27.9616
39	15.846	170.75	56.39	0.03	0.03	VB	3.0278
40	16.009	560.79	91.08	0.10	0.10	BV	6.1569
41	16.167	121.01	39.99	0.02	0.02	VB	3.0263
42	16.336	178.28	51.76	0.03	0.03	BV	3.4446
43	16.489	712.72	127.55	0.12	0.12	VB	5.5879
44	17.014	173.20	39.38	0.03	0.03	BB	4.3986
45	17.137	117.60	43.47	0.02	0.02	BB	2.7056

6/21/2013 12:41:08 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol003.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	17.469	158.00	35.93	0.03	0.03	BB	4.3969
47	17.611	119.20	41.71	0.02	0.02	BB	2.8579
48	17.777	66.00	28.18	0.01	0.01	BB	2.3420
49	17.963	328.00	58.57	0.06	0.06	BB	5.6005
50	18.100	84.00	43.82	0.01	0.01	BB	1.9171
51	19.178	2444.45	199.01	0.42	0.42	BV	12.2832
52	19.227	687.17	180.44	0.12	0.12	VV	3.8082
53	19.315	512.12	143.45	0.09	0.09	VV	3.5700
54	19.445	692.86	76.36	0.12	0.12	VB	9.0732
55	20.244	86.40	41.94	0.01	0.01	BB	2.0599
56	20.373	254.80	63.37	0.04	0.04	BB	4.0210
57	20.501	258.80	73.53	0.04	0.04	BB	3.5197
58	20.814	71.20	35.47	0.01	0.01	BB	2.0074
59	20.999	231.39	55.13	0.04	0.04	BV	4.1968
60	21.624	119898.61	3193.61	20.78	20.78	VB	37.5433
61	22.765	454.00	87.81	0.08	0.08	BB	5.1700
62	22.923	358.40	85.66	0.06	0.06	BB	4.1838
63	23.078	265.80	81.61	0.05	0.05	BB	3.2568
64	23.225	191.00	86.16	0.03	0.03	BB	2.2169
65	24.038	234.00	75.92	0.04	0.04	BB	3.0822
66	24.198	72.80	30.95	0.01	0.01	BB	2.3518
67	24.371	208.40	35.54	0.04	0.04	BB	5.8634
68	24.530	226.40	47.37	0.04	0.04	BB	4.7789
69	24.850	187.80	50.53	0.03	0.03	BB	3.7167
		576858.60	30540.59	100.00	100.00		

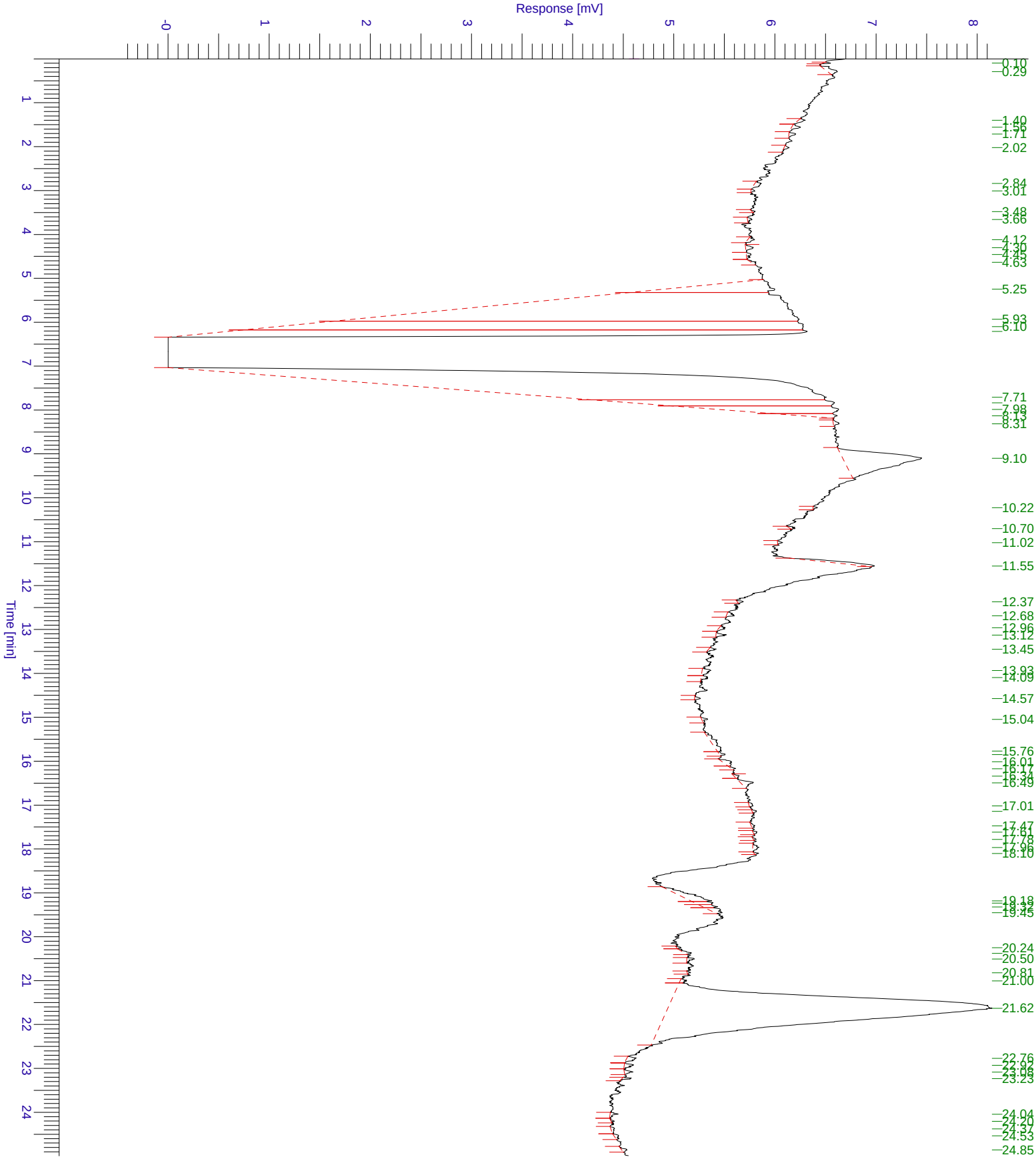
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : St1 Sample #: 003 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol003.raw
Date : 6/21/2013 12:41:53 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 2:12:29 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -0.41 mV High Point : 8.15 mV
Scale Factor: 1.0 Plot Offset: -0.41 mV Plot Scale: 8.6 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:42:55 PM
Operator	: rLuong	Sample Name	: St2
Sample Number	: 004	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/4
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 2:38:45 PM	Dilution Factor	: 1.00
		Cycle	: 4

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol004.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol004.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol004.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol004.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol004.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.218	265.20	52.85	0.12	0.12	BV	5.0177
2	0.393	379.91	46.28	0.17	0.17	VV	8.2083
3	0.524	276.69	63.17	0.13	0.13	VB	4.3800
4	0.663	94.00	40.72	0.04	0.04	BB	2.3083
5	0.868	274.01	59.50	0.13	0.13	BV	4.6049
6	1.006	396.37	78.12	0.18	0.18	VV	5.0741
7	1.141	192.74	68.24	0.09	0.09	VB	2.8243
8	1.307	153.30	46.22	0.07	0.07	BV	3.3168
9	1.467	247.90	56.79	0.11	0.11	VB	4.3653
10	1.697	324.00	57.26	0.15	0.15	BB	5.6589
11	2.275	116.00	42.06	0.05	0.05	BB	2.7577
12	2.917	144.80	53.48	0.07	0.07	BB	2.7077
13	3.237	260.80	49.89	0.12	0.12	BB	5.2276
14	4.353	255.40	59.94	0.12	0.12	BB	4.2610
15	5.466	56.60	32.34	0.03	0.03	BB	1.7503
16	5.670	159.40	51.17	0.07	0.07	BB	3.1150
17	5.803	145.20	60.18	0.07	0.07	BB	2.4126
18	7.894	105493.80	299.73	48.53	48.53	BB	351.9652
19	8.055	251.95	50.08	0.12	0.12	BV	5.0309
20	8.245	380.36	56.22	0.17	0.17	VV	6.7657
21	8.512	483.69	54.49	0.22	0.22	VB	8.8764
22	9.073	1786.80	175.11	0.82	0.82	BB	10.2036
23	9.819	120.80	34.74	0.06	0.06	BB	3.4770
24	10.139	128.80	32.23	0.06	0.06	BB	3.9969
25	10.471	121.40	42.27	0.06	0.06	BB	2.8718
26	10.678	187.60	37.49	0.09	0.09	BB	5.0045
27	10.942	76.00	42.89	0.03	0.03	BB	1.7719
28	11.577	3103.60	218.51	1.43	1.43	BB	14.2035
29	12.227	158.40	64.72	0.07	0.07	BB	2.4473
30	12.704	97.80	51.46	0.04	0.04	BB	1.9003
31	13.027	112.00	48.19	0.05	0.05	BB	2.3243
32	13.510	126.60	76.59	0.06	0.06	BB	1.6530
33	14.650	145.00	41.90	0.07	0.07	BB	3.4610
34	14.967	262.00	65.92	0.12	0.12	BB	3.9747
35	15.122	298.40	65.21	0.14	0.14	BB	4.5763
36	15.445	423.40	88.99	0.19	0.19	BB	4.7576
37	16.143	437.80	81.55	0.20	0.20	BB	5.3682
38	16.253	132.00	49.32	0.06	0.06	BB	2.6762
39	16.405	339.60	83.36	0.16	0.16	BB	4.0738
40	16.555	260.00	73.69	0.12	0.12	BB	3.5283
41	17.372	378.40	67.38	0.17	0.17	BB	5.6159
42	17.692	60.80	34.53	0.03	0.03	BB	1.7607
43	18.843	199.00	39.20	0.09	0.09	BB	5.0771
44	19.483	5002.68	136.42	2.30	2.30	BV	36.6712
45	19.634	220.92	52.61	0.10	0.10	VB	4.1990

6/21/2013 12:42:55 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol004.rst

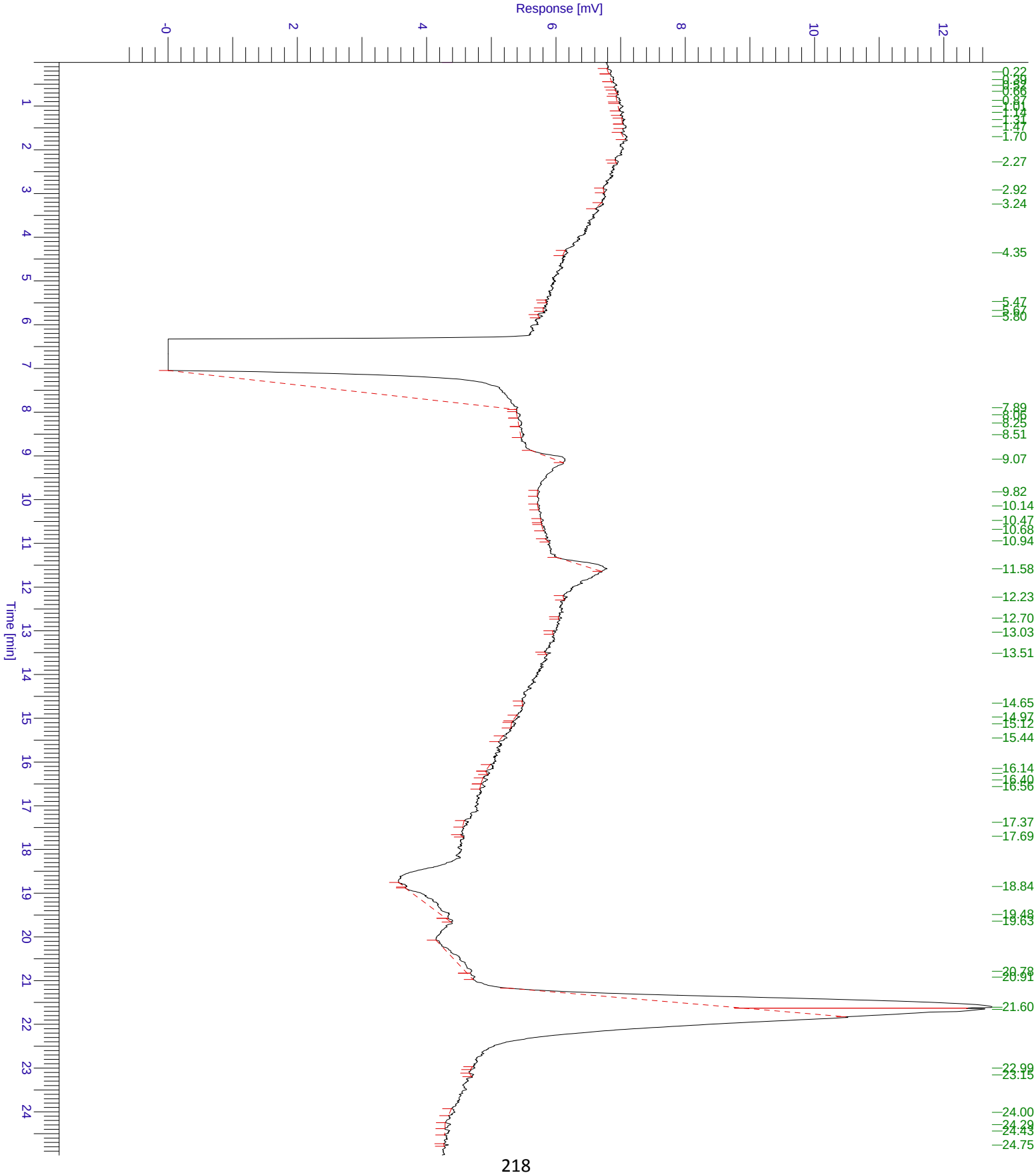
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	20.779	3334.96	107.42	1.53	1.53	BV	31.0455
47	20.913	370.24	69.21	0.17	0.17	VB	5.3493
48	21.601	63495.33	4061.43	29.21	29.21	BV	15.6337
49	21.649	24182.87	3580.62	11.12	11.12	VB	6.7538
50	22.992	121.20	48.98	0.06	0.06	BB	2.4743
51	23.149	127.40	52.48	0.06	0.06	BB	2.4274
52	24.005	423.40	76.11	0.19	0.19	BB	5.5630
53	24.286	360.43	83.51	0.17	0.17	BV	4.3159
54	24.433	339.97	77.04	0.16	0.16	VB	4.4128
55	24.755	96.40	62.25	0.04	0.04	BB	1.5486
		217384.12	11302.09	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : St2 Sample #: 004 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol004.raw
Date : 6/21/2013 12:43:27 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 2:38:45 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -0.64 mV High Point : 12.75 mV
Scale Factor: 1.0 Plot Offset: -0.64 mV Plot Scale: 13.4 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:44:19 PM
Operator	: rLuong	Sample Name	: St3
Sample Number	: 005	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/5
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 3:05:01 PM	Dilution Factor	: 1.00
		Cycle	: 5

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol005.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol005.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol005.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol005.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol005.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.135	199.60	52.14	0.02	0.02	BB	3.8285
2	0.270	341.20	84.41	0.03	0.03	BB	4.0422
3	0.939	500.00	78.52	0.04	0.04	BB	6.3674
4	1.071	139.40	41.89	0.01	0.01	BB	3.3279
5	1.235	287.58	66.39	0.02	0.02	BV	4.3316
6	1.380	212.67	56.18	0.02	0.02	VV	3.7853
7	1.547	273.35	78.14	0.02	0.02	VB	3.4979
8	2.009	151.00	33.03	0.01	0.01	BB	4.5717
9	2.210	192.80	42.71	0.02	0.02	BB	4.5143
10	2.508	70.20	35.60	0.01	0.01	BB	1.9717
11	2.668	163.43	43.07	0.01	0.01	BV	3.7949
12	3.473	167.73	30.32	0.01	0.01	VB	5.5329
13	4.290	1020.80	37.53	0.09	0.09	BB	27.1990
14	4.491	320.63	49.63	0.03	0.03	BV	6.4599
15	5.232	875.28	36.87	0.08	0.08	VB	23.7368
16	6.047	217.00	66.18	0.02	0.02	BB	3.2791
17	7.800	200857.73	2178.80	17.39	17.39	BV	92.1874
18	7.981	9856.67	871.85	0.85	0.85	VB	11.3055
19	9.089	19510.40	820.18	1.69	1.69	BE	23.7880
20	9.742	298.00	66.19	0.03	0.03	EB	4.5024
21	10.607	235.18	30.49	0.02	0.02	BV	7.7127
22	10.750	207.42	28.95	0.02	0.02	VB	7.1646
23	11.064	249.20	45.34	0.02	0.02	BB	5.4965
24	11.198	213.58	58.91	0.02	0.02	BV	3.6255
25	11.551	30927.02	1121.09	2.68	2.68	VB	27.5866
26	12.537	121.60	16.50	0.01	0.01	BB	7.3681
27	12.809	231.60	56.10	0.02	0.02	BB	4.1281
28	13.274	68.80	24.01	0.01	0.01	BB	2.8649
29	13.479	189.20	30.31	0.02	0.02	BB	6.2430
30	15.291	1760.00	40.24	0.15	0.15	BB	43.7350
31	16.318	392.60	68.45	0.03	0.03	BB	5.7356
32	17.313	6140.96	199.78	0.53	0.53	BV	30.7381
33	17.558	1993.99	156.58	0.17	0.17	VV	12.7346
34	17.688	559.14	71.22	0.05	0.05	VV	7.8515
35	17.806	407.90	91.38	0.04	0.04	VB	4.4636
36	17.931	212.81	83.59	0.02	0.02	BV	2.5459
37	18.051	326.02	105.58	0.03	0.03	VV	3.0880
38	18.175	712.41	129.04	0.06	0.06	VV	5.5208
39	18.299	553.96	88.58	0.05	0.05	VB	6.2536
40	18.949	3728.80	475.81	0.32	0.32	BB	7.8368
41	19.255	4649.19	539.13	0.40	0.40	BV	8.6236
42	19.282	1005.91	520.80	0.09	0.09	VV	1.9315
43	19.402	3704.65	437.52	0.32	0.32	VV	8.4674
44	19.528	1864.39	272.17	0.16	0.16	VV	6.8500
45	19.600	419.06	97.86	0.04	0.04	VB	4.2823

6/21/2013 12:44:19 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol005.rst

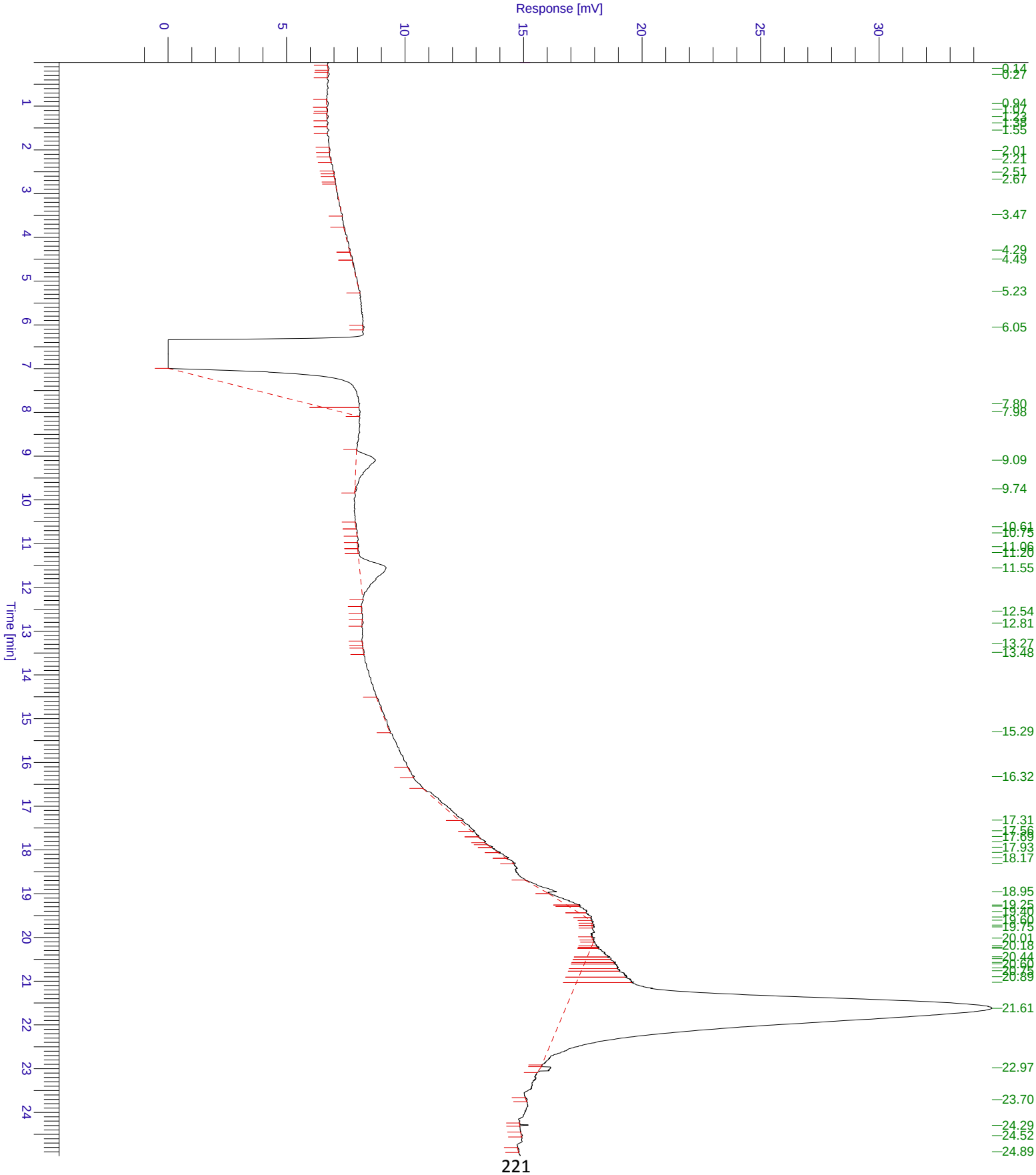
Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	19.712	170.82	85.85	0.01	0.01	BV	1.9898
47	19.752	245.58	94.28	0.02	0.02	VB	2.6049
48	20.010	272.00	119.21	0.02	0.02	BB	2.2818
49	20.180	630.82	189.77	0.05	0.05	BV	3.3241
50	20.216	500.52	304.46	0.04	0.04	VV	1.6439
51	20.240	458.89	329.56	0.04	0.04	VV	1.3924
52	20.436	7425.84	932.25	0.64	0.64	VV	7.9655
53	20.481	3359.15	1035.89	0.29	0.29	VV	3.2428
54	20.565	4839.36	1266.97	0.42	0.42	VV	3.8196
55	20.599	2521.23	1299.93	0.22	0.22	VV	1.9395
56	20.690	9005.62	1479.36	0.78	0.78	VV	6.0875
57	20.755	5469.03	1612.08	0.47	0.47	VV	3.3925
58	20.892	15159.40	2018.81	1.31	1.31	VV	7.5091
59	21.019	16420.82	2387.65	1.42	1.42	VV	6.8774
60	21.615	788525.31	17991.56	68.28	68.28	VB	43.8275
61	22.971	2414.60	471.54	0.21	0.21	BB	5.1206
62	23.700	188.80	62.96	0.02	0.02	BB	2.9989
63	24.287	328.00	302.98	0.03	0.03	BB	1.0826
64	24.521	168.00	51.70	0.01	0.01	BB	3.2496
65	24.889	213.60	42.81	0.02	0.02	BB	4.9900
		1154848.27	41638.67	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : St3 Sample #: 005 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol005.raw
Date : 6/21/2013 12:44:49 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 3:05:01 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -1.74 mV High Point : 34.77 mV
Scale Factor: 1.0 Plot Offset: -1.74 mV Plot Scale: 36.5 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:46:16 PM
Operator	: rLuong	Sample Name	: St4
Sample Number	: 006	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/6
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 3:31:17 PM	Dilution Factor	: 1.00
		Cycle	: 6

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol006.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol006.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol006.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol006.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol006.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.290	53.20	46.18	0.01	0.01	BB	1.1519
2	0.337	118.41	86.08	0.03	0.03	BV	1.3755
3	0.420	249.88	87.33	0.06	0.06	VV	2.8613
4	0.546	771.17	133.32	0.20	0.20	VV	5.7843
5	0.595	126.55	70.64	0.03	0.03	VB	1.7913
6	0.729	1068.72	228.59	0.27	0.27	BV	4.6753
7	0.863	2000.18	306.85	0.51	0.51	VV	6.5185
8	0.994	2557.84	314.52	0.65	0.65	VV	8.1325
9	1.055	795.29	264.31	0.20	0.20	VV	3.0089
10	1.129	589.44	212.24	0.15	0.15	VV	2.7772
11	1.208	600.57	148.18	0.15	0.15	VV	4.0529
12	1.252	284.72	160.11	0.07	0.07	VV	1.7783
13	1.291	318.05	113.65	0.08	0.08	VB	2.7984
14	1.411	135.20	56.31	0.03	0.03	BB	2.4008
15	1.468	189.20	93.77	0.05	0.05	BB	2.0178
16	1.516	81.80	65.85	0.02	0.02	BB	1.2422
17	1.607	138.40	107.99	0.04	0.04	BB	1.2816
18	1.649	104.40	69.88	0.03	0.03	BB	1.4940
19	1.781	157.04	114.72	0.04	0.04	BV	1.3688
20	1.852	441.55	173.21	0.11	0.11	VV	2.5492
21	1.947	965.51	196.41	0.25	0.25	VB	4.9157
22	2.130	355.23	116.06	0.09	0.09	BV	3.0608
23	2.168	208.77	98.78	0.05	0.05	VB	2.1136
24	2.265	168.80	112.14	0.04	0.04	BB	1.5052
25	2.982	143.92	60.54	0.04	0.04	BV	2.3774
26	3.055	193.88	106.47	0.05	0.05	VB	1.8210
27	3.213	58.00	52.57	0.01	0.01	BB	1.1032
28	3.248	96.60	70.71	0.02	0.02	BB	1.3661
29	3.384	250.40	101.64	0.06	0.06	BB	2.4635
30	3.434	46.00	45.53	0.01	0.01	BB	1.0102
31	3.535	139.60	153.21	0.04	0.04	BB	0.9112
32	4.534	168.20	55.55	0.04	0.04	BB	3.0281
33	4.593	91.60	91.59	0.02	0.02	BB	1.0001
34	4.953	756.00	248.92	0.19	0.19	BB	3.0371
35	5.158	337.60	113.43	0.09	0.09	BB	2.9764
36	5.298	43.20	57.48	0.01	0.01	BB	0.7515
37	5.629	989.00	211.40	0.25	0.25	BB	4.6784
38	5.749	59.80	72.91	0.02	0.02	BB	0.8202
39	6.180	1777.60	56.88	0.45	0.45	BB	31.2493
40	7.352	91975.08	4281.19	23.41	23.41	BV	21.4835
41	7.423	12884.63	3760.88	3.28	3.28	VV	3.4260
42	7.643	33411.07	2368.09	8.50	8.50	VV	14.1089
43	7.717	11156.70	1800.97	2.84	2.84	VV	6.1948
44	7.875	1492.90	490.49	0.38	0.38	VB	3.0437
45	8.046	544.80	116.69	0.14	0.14	BB	4.6687

6/21/2013 12:46:16 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol006.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	8.339	317.40	29.38	0.08	0.08	BB	10.8045
47	9.011	3317.60	360.10	0.84	0.84	BB	9.2131
48	9.237	615.60	259.20	0.16	0.16	BB	2.3750
49	10.469	153.00	49.15	0.04	0.04	BB	3.1129
50	10.796	371.20	62.28	0.09	0.09	BB	5.9600
51	11.546	4693.40	327.56	1.19	1.19	BB	14.3282
52	12.263	252.80	56.53	0.06	0.06	BB	4.4722
53	12.442	498.20	78.52	0.13	0.13	BB	6.3447
54	12.803	50.06	50.56	0.01	0.01	BV	0.9901
55	12.835	92.53	78.28	0.02	0.02	VV	1.1821
56	12.860	216.41	82.58	0.06	0.06	VV	2.6207
57	12.954	167.01	62.44	0.04	0.04	VB	2.6747
58	13.070	158.00	76.87	0.04	0.04	BB	2.0554
59	13.200	219.20	76.41	0.06	0.06	BB	2.8686
60	13.282	50.00	53.91	0.01	0.01	BB	0.9274
61	13.550	503.20	111.12	0.13	0.13	BB	4.5286
62	13.673	325.20	87.19	0.08	0.08	BB	3.7300
63	15.093	630.80	123.81	0.16	0.16	BB	5.0950
64	15.750	180.00	59.31	0.05	0.05	BB	3.0350
65	16.417	201.80	42.68	0.05	0.05	BB	4.7282
66	16.551	35.00	38.72	0.01	0.01	BB	0.9039
67	17.030	1664.40	72.52	0.42	0.42	BB	22.9493
68	18.008	9272.62	794.42	2.36	2.36	BV	11.6722
69	18.180	29641.78	1203.30	7.54	7.54	VB	24.6337
70	21.593	170177.60	9402.00	43.31	43.31	BB	18.1001
		392901.28	31333.10	100.00	100.00		

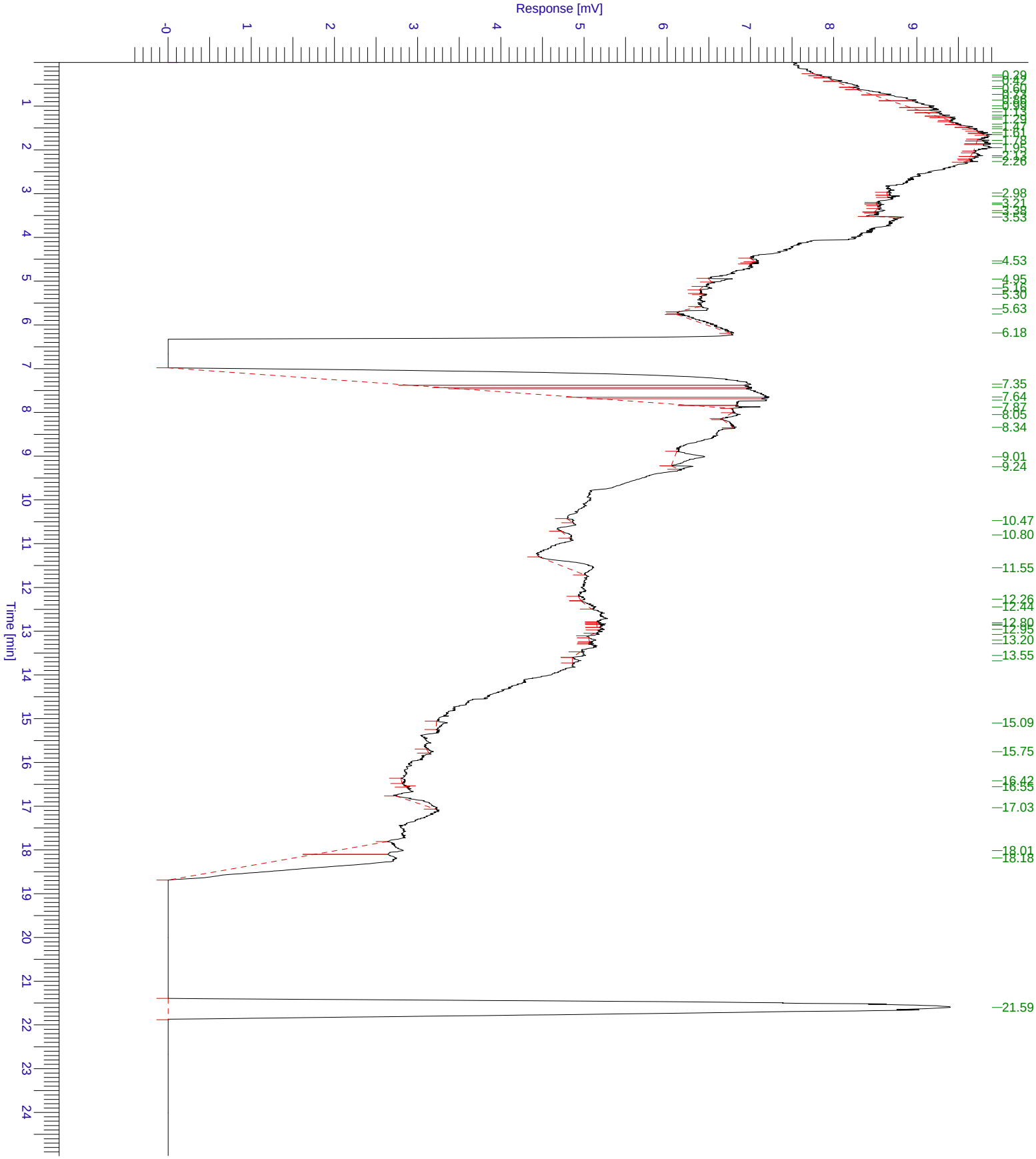
Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : St4 Sample #: 006 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol006.raw
Date : 6/21/2013 12:46:34 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 3:31:17 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -0.49 mV High Point : 9.91 mV
Scale Factor: 1.0 Plot Offset: -0.49 mV Plot Scale: 10.4 mV



Software Version	: 6.3.2.0646	Date	: 6/21/2013 12:47:01 PM
Operator	: rLuong	Sample Name	: St5
Sample Number	: 007	Study	: Ethanol
AutoSampler	: SER200	Rack/Vial	: 1/7
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 24.99 min
Sampling Rate	: 2.5000 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 0.000000
Data Acquisition Time	: 6/11/2013 3:57:33 PM	Dilution Factor	: 1.00
		Cycle	: 7

Raw Data File : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol007.raw
Result File : c:\hplc data\robin\ethanol_june 10-2013\ethanol007.rst [Editing in Progress]
Inst Method : C:\HPLC Data\robin\Method Robin 87H from C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol007.raw
Proc Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol007.rst [Editing in Progress]
Calib Method : C:\HPLC Data\robin\Method Robin 87H from c:\hplc data\robin\ethanol_june 10-2013\ethanol007.rst [Editing in Progress]
Report Format File: C:\HPLC Data\robin\Method Robin 87H.rpt
Sequence File : C:\HPLC Data\robin\Valera Sequence 87H-Etanol_June 10_2013.seq

DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
-	0.001	0.00	0.00	0.00	0.00		----------
1	0.100	354.00	122.61	0.01	0.01	BB	2.8872
2	0.158	290.00	123.00	0.01	0.01	BB	2.3577
3	0.244	365.20	57.49	0.01	0.01	BB	6.3528
4	0.362	170.80	72.21	0.01	0.01	BB	2.3652
5	0.418	351.69	148.13	0.01	0.01	BV	2.3742
6	0.493	153.31	116.28	0.00	0.00	VB	1.3185
7	0.544	72.80	66.95	0.00	0.00	BB	1.0873
8	0.625	128.80	111.25	0.00	0.00	BB	1.1578
9	0.678	296.40	123.12	0.01	0.01	BB	2.4073
10	0.758	208.40	113.05	0.01	0.01	BB	1.8435
11	0.804	77.20	69.43	0.00	0.00	BB	1.1120
12	0.892	806.00	159.83	0.02	0.02	BB	5.0429
13	1.178	140.29	84.58	0.00	0.00	BV	1.6586
14	1.218	275.70	132.46	0.01	0.01	VB	2.0814
15	1.403	282.40	75.14	0.01	0.01	BB	3.7582
16	1.489	148.40	95.91	0.00	0.00	BB	1.5473
17	1.573	232.40	89.66	0.01	0.01	BB	2.5919
18	1.630	213.24	184.63	0.01	0.01	BV	1.1549
19	1.656	317.67	166.35	0.01	0.01	VV	1.9097
20	1.701	420.47	162.51	0.01	0.01	VV	2.5873
21	1.784	560.35	117.20	0.02	0.02	VV	4.7813
22	1.857	142.96	107.99	0.00	0.00	VV	1.3238
23	1.888	128.31	103.88	0.00	0.00	VB	1.2352
24	2.033	114.40	60.54	0.00	0.00	BB	1.8896
25	2.176	65.60	61.79	0.00	0.00	BB	1.0617
26	2.287	329.82	117.16	0.01	0.01	BV	2.8151
27	2.358	336.58	94.51	0.01	0.01	VB	3.5613
28	2.451	166.01	101.64	0.01	0.01	BV	1.6333
29	2.523	157.59	79.99	0.00	0.00	VB	1.9702
30	2.624	452.00	99.00	0.01	0.01	BB	4.5657
31	2.833	346.03	76.90	0.01	0.01	BV	4.5000
32	2.922	96.72	78.37	0.00	0.00	VV	1.2340
33	2.953	84.05	76.53	0.00	0.00	VB	1.0983
34	2.986	173.20	103.24	0.01	0.01	BB	1.6777
35	3.056	89.20	68.23	0.00	0.00	BB	1.3074
36	3.176	472.60	107.22	0.01	0.01	BB	4.4078
37	3.322	111.80	81.39	0.00	0.00	BB	1.3737
38	3.413	57.12	25.88	0.00	0.00	BV	2.2072
39	3.474	103.68	51.40	0.00	0.00	VB	2.0173
40	3.642	136.40	40.68	0.00	0.00	BB	3.3533
41	3.783	76.60	71.99	0.00	0.00	BB	1.0640
42	3.842	71.40	48.21	0.00	0.00	BB	1.4810
43	3.981	61.20	62.00	0.00	0.00	BB	0.9871
44	4.009	96.60	93.92	0.00	0.00	BB	1.0285
45	4.108	114.40	65.75	0.00	0.00	BB	1.7400

6/21/2013 12:47:01 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol007.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
46	4.208	57.76	56.14	0.00	0.00	BV	1.0288
47	4.261	386.04	86.76	0.01	0.01	VB	4.4496
48	4.475	268.80	81.16	0.01	0.01	BB	3.3120
49	4.703	268.60	107.73	0.01	0.01	BB	2.4933
50	4.970	210.20	50.49	0.01	0.01	BB	4.1634
51	5.415	166.40	61.23	0.01	0.01	BB	2.7176
52	6.200	38076.60	2067.58	1.15	1.15	BB	18.4161
53	7.505	174466.02	3923.52	5.25	5.25	BV	44.4667
54	7.635	24377.53	2438.47	0.73	0.73	VV	9.9971
55	7.718	6990.32	1434.87	0.21	0.21	VV	4.8718
56	7.764	2550.41	932.51	0.08	0.08	VV	2.7350
57	7.822	1124.54	255.61	0.03	0.03	VB	4.3994
58	7.965	285.60	102.23	0.01	0.01	BB	2.7936
59	8.025	119.30	76.57	0.00	0.00	BV	1.5581
60	8.069	107.85	72.03	0.00	0.00	VV	1.4973
61	8.158	278.33	78.54	0.01	0.01	VV	3.5438
62	8.207	41.33	39.73	0.00	0.00	VB	1.0401
63	8.294	325.33	163.98	0.01	0.01	BV	1.9840
64	8.375	283.47	87.80	0.01	0.01	VB	3.2287
65	8.410	46.60	48.06	0.00	0.00	BB	0.9696
66	8.472	56.40	36.37	0.00	0.00	BB	1.5506
67	8.559	260.40	117.44	0.01	0.01	BB	2.2173
68	8.694	199.20	86.12	0.01	0.01	BB	2.3131
69	8.772	202.40	100.04	0.01	0.01	BB	2.0231
70	8.914	262.00	82.57	0.01	0.01	BB	3.1731
71	8.959	329.67	181.27	0.01	0.01	BV	1.8186
72	9.052	731.93	105.07	0.02	0.02	VB	6.9658
73	9.262	605.00	128.68	0.02	0.02	BB	4.7017
74	9.340	80.02	75.21	0.00	0.00	BV	1.0639
75	9.367	74.38	72.19	0.00	0.00	VB	1.0302
76	9.401	478.40	113.65	0.01	0.01	BB	4.2093
77	9.620	178.00	122.27	0.01	0.01	BB	1.4558
78	9.759	239.20	69.10	0.01	0.01	BB	3.4619
79	9.889	98.40	82.66	0.00	0.00	BB	1.1905
80	10.160	76.60	79.61	0.00	0.00	BB	0.9622
81	10.451	43.60	60.63	0.00	0.00	BB	0.7192
82	10.533	132.80	85.45	0.00	0.00	BB	1.5541
83	10.563	376.21	109.57	0.01	0.01	BV	3.4336
84	10.702	226.98	87.54	0.01	0.01	VV	2.5929
85	10.768	253.21	70.40	0.01	0.01	VB	3.5966
86	10.834	216.30	87.73	0.01	0.01	BV	2.4655
87	10.907	129.70	50.75	0.00	0.00	VB	2.5558
88	11.110	195.60	66.30	0.01	0.01	BB	2.9500
89	11.186	222.52	107.14	0.01	0.01	BV	2.0768
90	11.239	295.48	112.03	0.01	0.01	VB	2.6374
91	11.511	1856.00	251.41	0.06	0.06	BB	7.3825
92	11.649	357.60	95.46	0.01	0.01	BB	3.7461
93	11.917	137.40	75.77	0.00	0.00	BB	1.8134
94	12.189	148.21	69.64	0.00	0.00	BV	2.1282
95	12.243	104.99	76.33	0.00	0.00	VB	1.3755
96	12.481	583.80	101.03	0.02	0.02	BB	5.7783
97	12.615	232.60	59.35	0.01	0.01	BB	3.9189
98	12.759	540.60	95.33	0.02	0.02	BB	5.6708
99	12.907	584.39	119.65	0.02	0.02	BV	4.8842
100	13.007	144.01	75.57	0.00	0.00	VB	1.9057
101	13.128	248.40	52.60	0.01	0.01	BB	4.7227
102	13.215	95.60	76.58	0.00	0.00	BB	1.2483
103	13.523	136.76	71.72	0.00	0.00	BV	1.9069
104	13.588	60.44	53.74	0.00	0.00	VB	1.1247
105	13.641	49.40	49.02	0.00	0.00	BB	1.0077
106	13.956	51.20	49.22	0.00	0.00	BB	1.0402
107	14.071	242.40	93.32	0.01	0.01	BB	2.5976
108	14.157	146.09	56.12	0.00	0.00	BV	2.6032
109	14.230	464.71	99.32	0.01	0.01	VB	4.6788
110	14.415	356.00	73.43	0.01	0.01	BB	4.8484
111	14.555	338.67	109.91	0.01	0.01	BV	3.0813
112	14.604	245.73	118.40	0.01	0.01	VB	2.0755
113	15.218	221.60	60.99	0.01	0.01	BB	3.6335
114	15.251	63.60	54.36	0.00	0.00	BB	1.1701

6/21/2013 12:47:01 PM Result: c:\hplc data\robin\ethanol_june 10-2013\ethanol007.rst

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
115	15.368	181.20	68.39	0.01	0.01	BB	2.6494
116	15.517	264.00	89.09	0.01	0.01	BB	2.9632
117	15.826	590.60	124.98	0.02	0.02	BB	4.7257
118	15.989	126.20	48.25	0.00	0.00	BB	2.6154
119	16.223	259.60	39.14	0.01	0.01	BB	6.6318
120	16.322	107.60	42.96	0.00	0.00	BB	2.5047
121	16.636	86.40	47.74	0.00	0.00	BB	1.8099
122	16.837	263.60	39.39	0.01	0.01	BB	6.6918
123	16.968	289.20	61.12	0.01	0.01	BB	4.7319
124	17.159	203.20	41.20	0.01	0.01	BB	4.9324
125	17.286	146.40	55.12	0.00	0.00	BB	2.6559
126	17.430	91.80	51.77	0.00	0.00	BB	1.7731
127	17.761	147.00	51.34	0.00	0.00	BB	2.8630
128	17.822	71.60	44.59	0.00	0.00	BB	1.6059
129	18.085	171.40	83.19	0.01	0.01	BB	2.0602
130	18.658	104.83	62.72	0.00	0.00	BV	1.6716
131	18.744	219.37	61.29	0.01	0.01	VB	3.5789
132	18.843	202.00	40.47	0.01	0.01	BB	4.9914
133	18.883	159.22	98.73	0.00	0.00	BV	1.6127
134	18.962	321.34	111.00	0.01	0.01	VV	2.8950
135	18.987	118.44	111.57	0.00	0.00	VV	1.0615
136	19.105	1024.22	149.35	0.03	0.03	VV	6.8581
137	19.155	368.17	143.09	0.01	0.01	VV	2.5730
138	19.246	726.98	130.85	0.02	0.02	VV	5.5560
139	19.285	359.23	162.54	0.01	0.01	VV	2.2101
140	19.369	353.18	103.37	0.01	0.01	VV	3.4165
141	19.423	197.43	80.21	0.01	0.01	VB	2.4614
142	19.555	275.17	71.70	0.01	0.01	BV	3.8380
143	19.690	586.00	164.62	0.02	0.02	VB	3.5597
144	19.833	159.94	97.13	0.00	0.00	BV	1.6467
145	19.867	226.06	81.62	0.01	0.01	VB	2.7696
146	20.007	84.80	62.97	0.00	0.00	BB	1.3466
147	20.175	200.02	66.88	0.01	0.01	BV	2.9906
148	20.364	195.12	71.22	0.01	0.01	VB	2.7395
149	20.504	182.15	72.48	0.01	0.01	BV	2.5131
150	20.556	85.25	50.58	0.00	0.00	VB	1.6855
151	20.637	130.40	82.69	0.00	0.00	BB	1.5770
152	20.771	81.18	70.29	0.00	0.00	BV	1.1550
153	20.831	730.99	145.77	0.02	0.02	VV	5.0146
154	21.582	3027716.93	76668.46	91.20	91.20	VV	39.4910
155	22.862	262.30	102.82	0.01	0.01	VB	2.5509
156	23.011	101.20	46.84	0.00	0.00	BB	2.1606
157	23.144	212.28	87.02	0.01	0.01	BV	2.4394
158	23.228	120.03	75.39	0.00	0.00	VV	1.5920
159	23.281	299.69	78.94	0.01	0.01	VB	3.7966
160	23.445	280.60	68.22	0.01	0.01	BB	4.1133
161	23.567	256.00	72.13	0.01	0.01	BB	3.5489
162	23.636	1564.20	442.18	0.05	0.05	BV	3.5375
163	23.701	2834.59	447.88	0.09	0.09	VV	6.3290
164	23.844	1821.14	288.87	0.05	0.05	VV	6.3044
165	24.026	113.67	37.91	0.00	0.00	VB	2.9989
166	24.176	158.40	56.39	0.00	0.00	BB	2.8088
167	24.344	169.20	70.92	0.01	0.01	BB	2.3857
168	24.620	69.00	41.07	0.00	0.00	BB	1.6800
169	24.863	278.80	50.07	0.01	0.01	BB	5.5678
		3320004.71	102579.61	100.00	100.00		

Warning -- Signal level out-of-range in peak

Missing Component Report

Component	Expected Retention (Calibration File)
standards	0.001

Chromatogram

Sample Name : St5 Sample #: 007 Page 1 of 1
FileName : C:\HPLC Data\robin\Ethanol_June 10-2013\Ethanol007.raw
Date : 6/21/2013 12:47:23 PM
Method : Method Robin 87H Time of Injection: 6/11/2013 3:57:33 PM
Start Time : 0.00 min End Time : 24.99 min Low Point : -4.57 mV High Point : 91.44 mV
Scale Factor: 1.0 Plot Offset: -4.57 mV Plot Scale: 96.0 mV

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