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## **APPENDICES**

## APPENDIX A

### Culture media

#### 1. Yeast Peptone Dextrose (YPD) Agar

Yeast extract	10	g
Bacto peptone	20	g
Glucose	20	g
Agar	18	g
Distilled water	1000	ml

Adjusted pH 4.5

Sterile by autoclaving at 121°C, 115 lb/in<sup>2</sup> for 15 min.

## APPENDIX B

### Reagents and Buffers

#### 1. Determination of reducing sugar

The reducing sugar was measured by the method of Somogyi (1952) using glucose as authentic sugar.

##### 1.1 Somogyi-Nelson Reagent

###### A. Alkaline Copper Reagent :

- Potassium sodium tartate (Rochelle salts) 40 g in 300 ml distilled water
- Disodium hydrogen phosphate dodecahydrate 71 g in 300 ml distilled water
- 10% Copper (II) sulfate 80 ml  
(8 g Copper (II) sulfate in 80 ml distilled water)
- 1N Sodium hydroxide 100 ml  
(4 g Sodium hydroxide in 100 ml distilled water)
- Sodium sulfate        180        g

Dissolve the solutions above and make up volume to 1000 ml.

###### B. Nelson Reagent

- Ammonium molybdate 53.2 g in 500 ml distilled water
- Sulfuric acid (conc.) 21        ml  
Sulfuric acid (conc.) is added into the ammonium molybdate.
- Sodium arsenate 6 g in 50 ml distilled water

Dissolve the solutions above and make up volume to 1000 ml.

##### 1.2 Procedure

- Put proper dilution of sample (1 ml) in a test tube
- Add Alkaline Copper solution (1 ml) and place in boiling water for 15 minutes.

Immediately cool in ice water.

- After addition of 1 ml Nelson solution, incubate at room temperature for 30 minutes and dilute by adding 5 ml of distilled water.

- Absorbance of samples was measured at 520 nm. Concentrations of the samples were compared to the standard curve for determination of values. Distilled water was used instead of sample as a blank.

### **1.3 Preparation of standard curve of glucose**

Glucose standard solutions (1 mg/ml) are prepared in distilled water. Standards of 0, 20, 40, 60, 80, 100, 120, 150, 180 and 200 µg/ml were prepared from glucose solution. The reactions were carried out with the same procedure as described previously. Absorbances were plotted against concentrations of standards.

### **1.4 Calculation of reducing sugar**

$$\text{Formula} \quad \text{Reducing sugar (g/l)} = \frac{A_{520} \times \text{dilution}}{\text{Slope}}$$

### **2. 0.1 M Citrate buffer pH 4.5**

Citric acid monohydrate (0.1 M) 21.01 g/l

Trisodium citrate dehydrate (0.1 M) 29.41 g/l

Mix 0.1 M citric acid (47 ml) with 0.1 M trisodium citrate (53 ml). Adjust pH to 4.5.

### **3. 0.1 M Citrate buffer pH 5.0**

Citric acid monohydrate (0.1 M) 21.01 g/l

Trisodium citrate dehydrate (0.1 M) 29.41 g/l

Mix 0.1 M citric acid (35 ml) with 0.1 M trisodium citrate (65 ml). Adjust pH to 5.0.

### **4. 0.1 M Citrate buffer pH 5.5**

Citric acid monohydrate (0.1 M) 21.01 g/l

Trisodium citrate dehydrate (0.1 M) 29.41 g/l

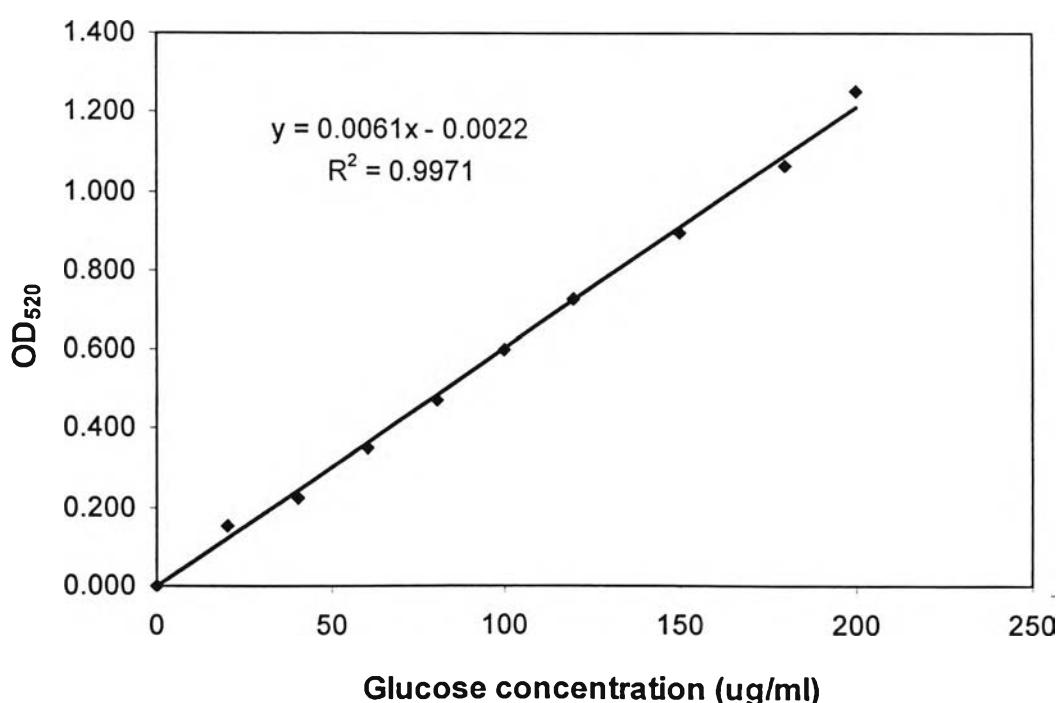
Mix 0.1 M citric acid (23.25 ml) with 0.1 M trisodium citrate (76.75 ml). Adjust pH to 5.5

## APPENDIX C

### Standard curve

#### C.1 Standard curve of glucose

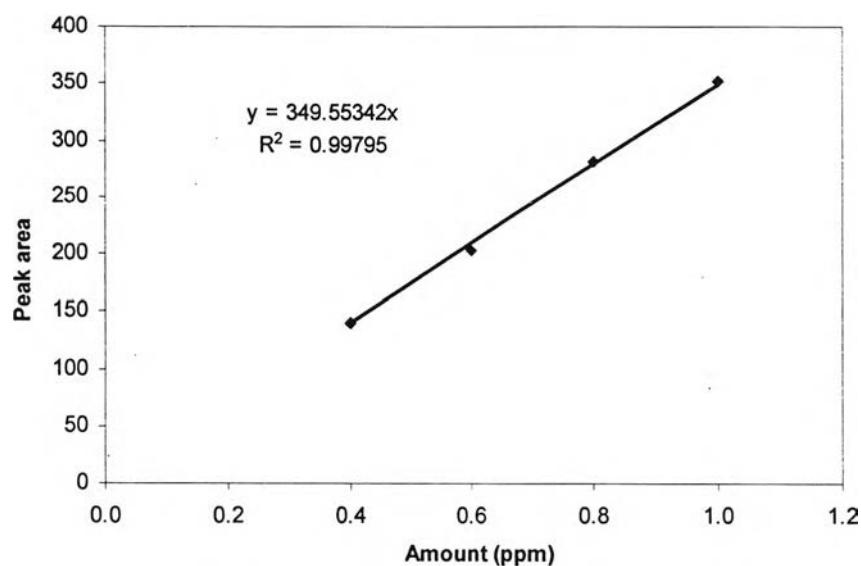
Glucose (ug/ml)	OD <sub>520</sub>	OD <sub>520</sub>	OD <sub>520</sub>	Avg OD <sub>520</sub>
0	0.000	0.000	0.000	0.000
20	0.116	0.183	0.159	0.153
40	0.228	0.219	0.224	0.224
60	0.352	0.353	0.353	0.353
80	0.473	0.473	0.476	0.474
100	0.610	0.624	0.568	0.601
120	0.727	0.727	0.802	0.727
150	0.865	0.890	0.929	0.895
180	1.058	1.065	1.045	1.062
200	1.248	1.258	1.420	1.253



## C.2 Sugar and byproducts in Ca(OH)<sub>2</sub> pretreatment hydrolysate

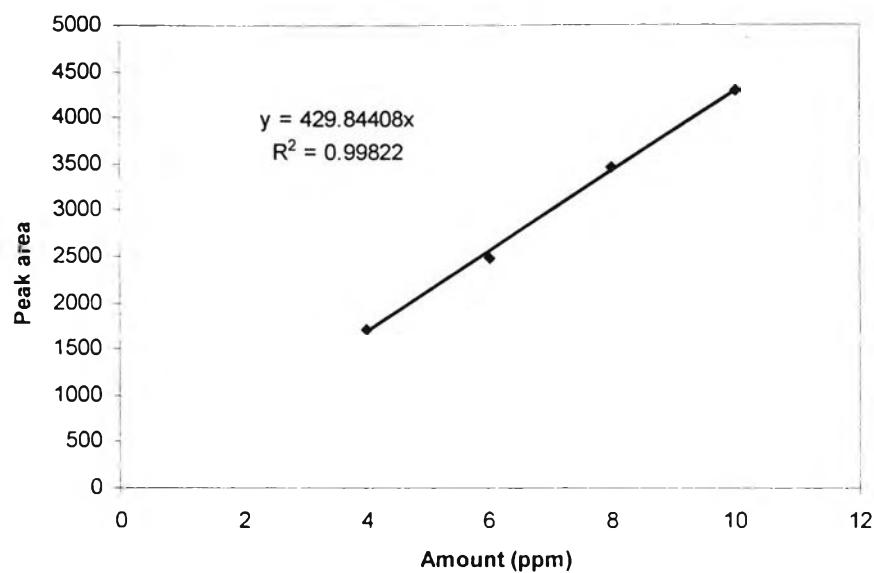
### C.2.1 Standard curve of hydroxymethyl furfural

Retention time (min)	Amount (ppm)	Area	Name
5.025	0.4	139.94321	Hydroxymethyl furfural (HMF)
	0.6	203.15800	
	0.8	281.57150	
	1.0	351.90610	



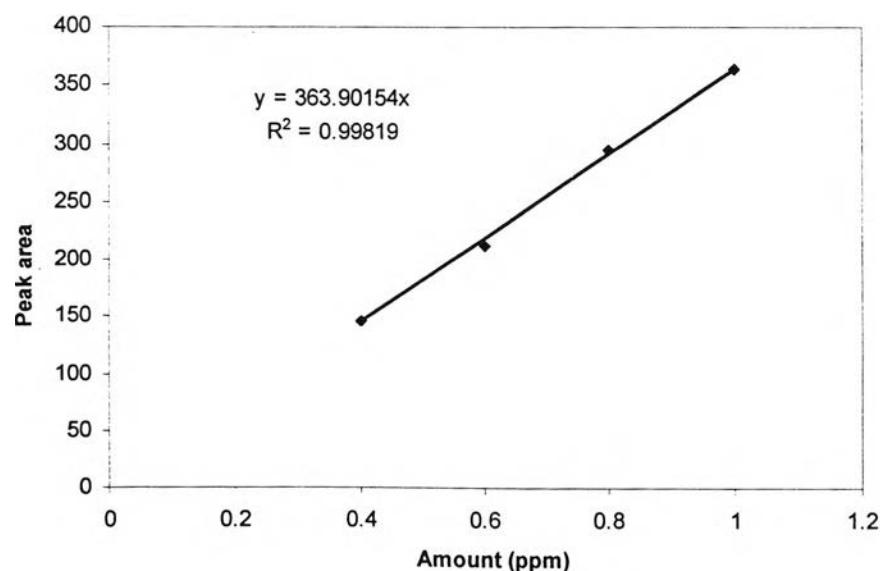
### C.2.2 Standard curve of furfural

Retention time (min)	Amount (ppm)	Area	Name
6.552	4	1723.07947	Furfural
	6	2509.15210	
	8	3481.65259	
	10	4304.58691	



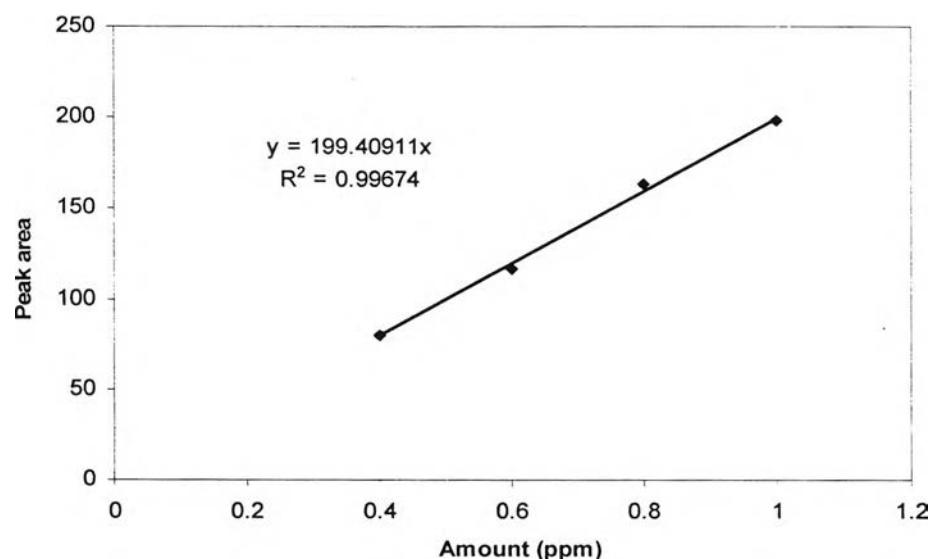
### C.2.3 Standard curve of 4-hydrobenzaldehyde

Retention time (min)	Amount (ppm)	Area	Name
11.027	0.4	146.22147	4-Hydrobenzaldehyde
	0.6	212.34325	
	0.8	294.66772	
	1.0	364.39862	



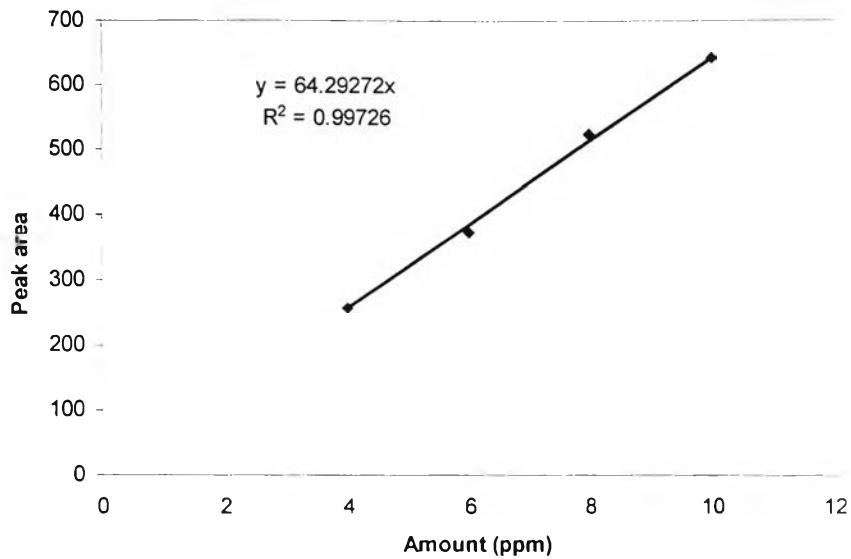
#### C.2.4 Standard curve of vanillin

Retention time (min)	Amount (ppm)	Area	Name
12.706	0.4	79.47014	Vanillin
	0.6	116.40575	
	0.8	163.40186	
	1.0	198.37068	



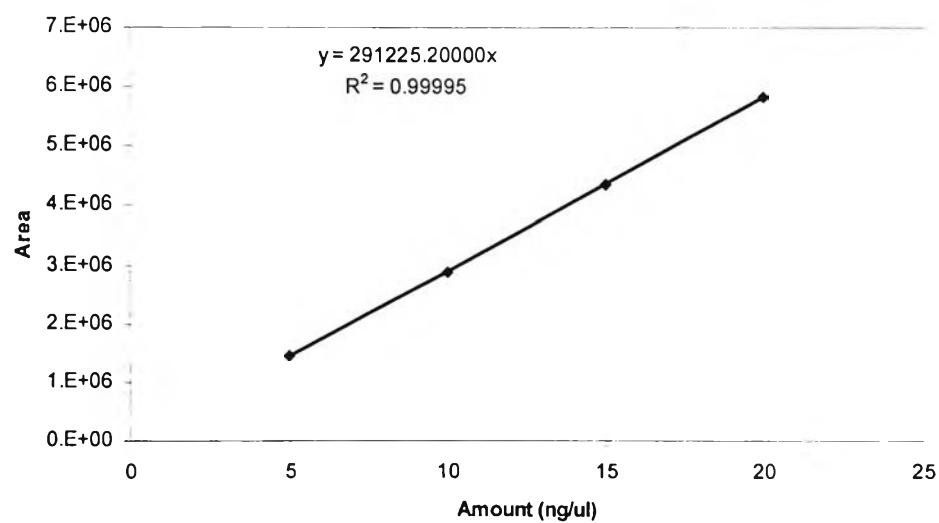
#### C.2.5 Standard curve of syringaldehyde

Retention time (min)	Amount (ppm)	Area	Name
13.475	4	257.13474	Syringaldehyde
	6	373.95203	
	8	524.03467	
	10	642.26984	



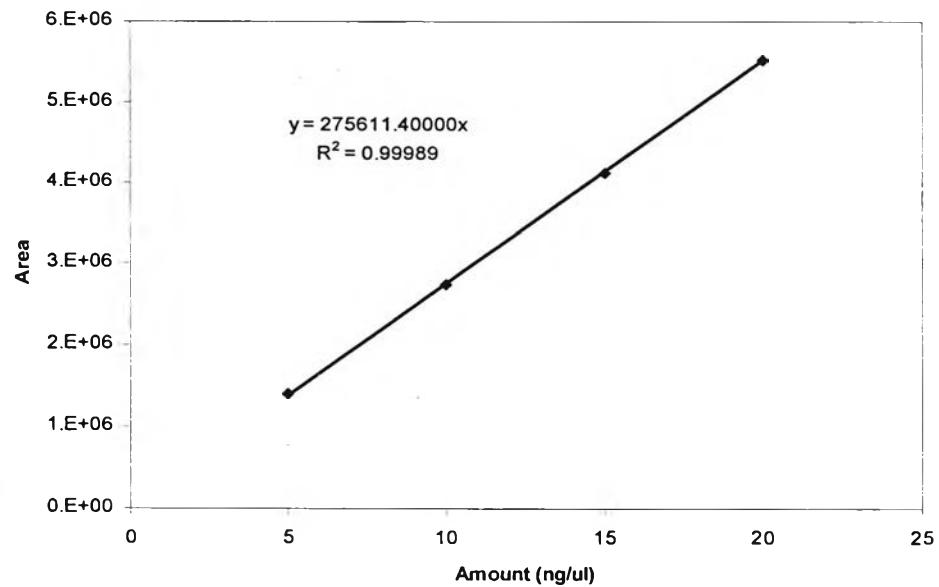
#### C.2.6 Standard curve of glucose

Retention time (min)	Amount (ng/uL)	Area	Name
12.327	5	1.46854e6	Glucose
	10	2.89263e6	
	15	4.37194e6	
	20	5.82854e6	



### C.2.7 Standard curve of xylose

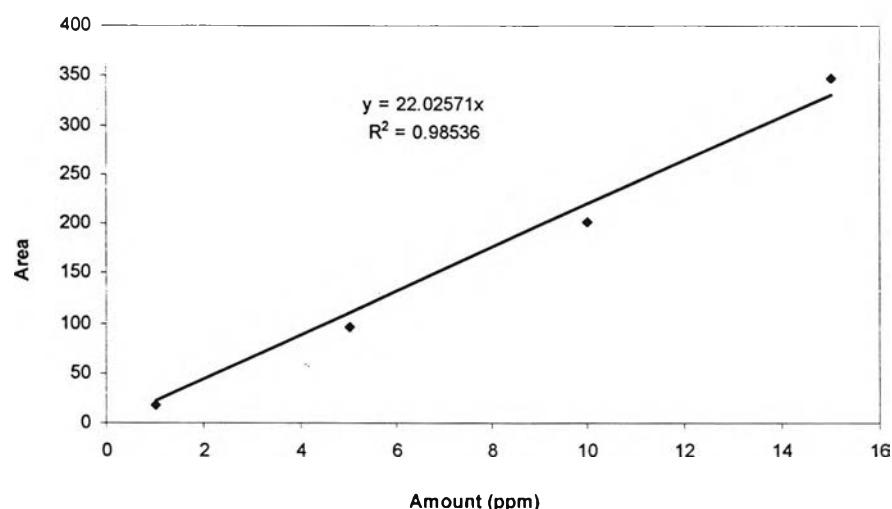
Retention time (min)	Amount (ng/uL)	Area	Name
13.479	5	1.39733e6	xylose
	10	2.73649e6	
	15	4.12452e6	
	20	5.52446e6	



### C.3 Sugar and byproducts in Ca(OH)<sub>2</sub> pretreatment hydrolysate

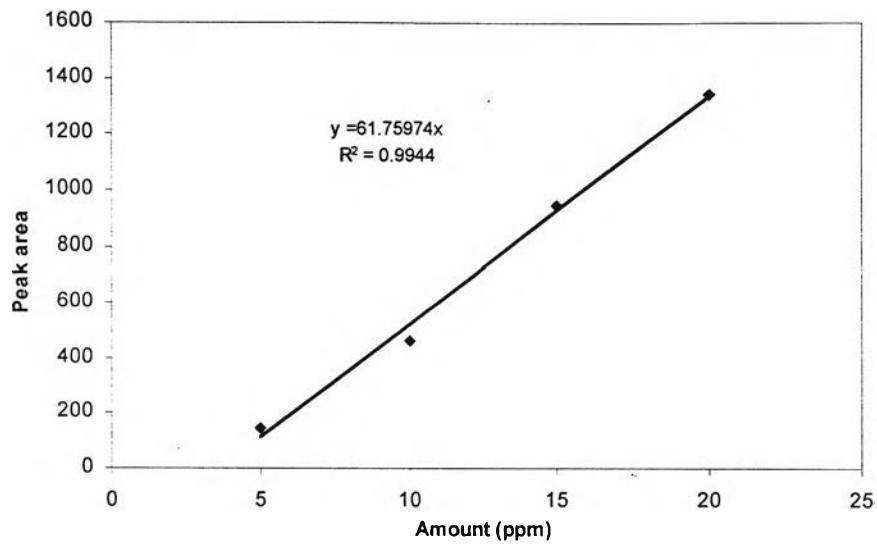
#### C.3.1 Standard curve of hydroxymethyl furfural

Retention time (min)	Amount (ppm)	Area	Name
5.062	1	17.74108	Hydroxymethyl furfural (HMF)
	5	95.89160	
	10	201.22179	
	15	348.10709	



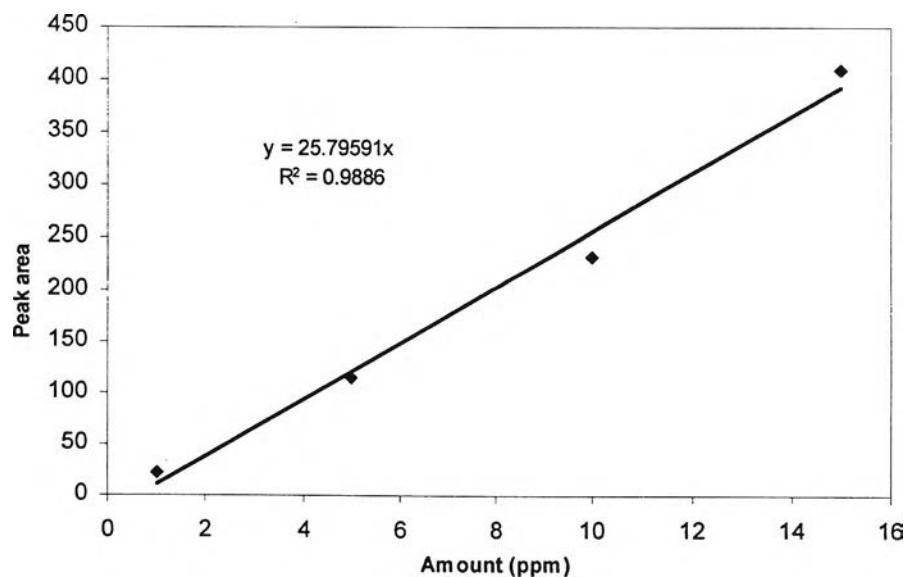
#### C.3.2 Standard curve of furfural

Retention time (min)	Amount (ppm)	Area	Name
6.748	5	147.05643	Furfural
	10	463.12479	
	15	940.23602	
	20	1342.48657	



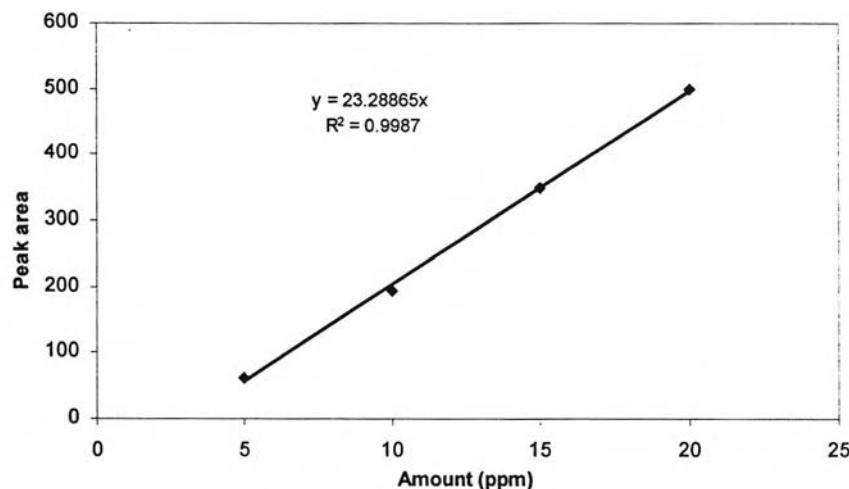
### C.3.3 Standard curve of 4-hydrobenzaldehyde

Retention time (min)	Amount (ppm)	Area	Name
10.980	1	21.66099	4-Hydrobenzaldehyde
	5	115.07211	
	10	232.68480	
	15	408.69968	



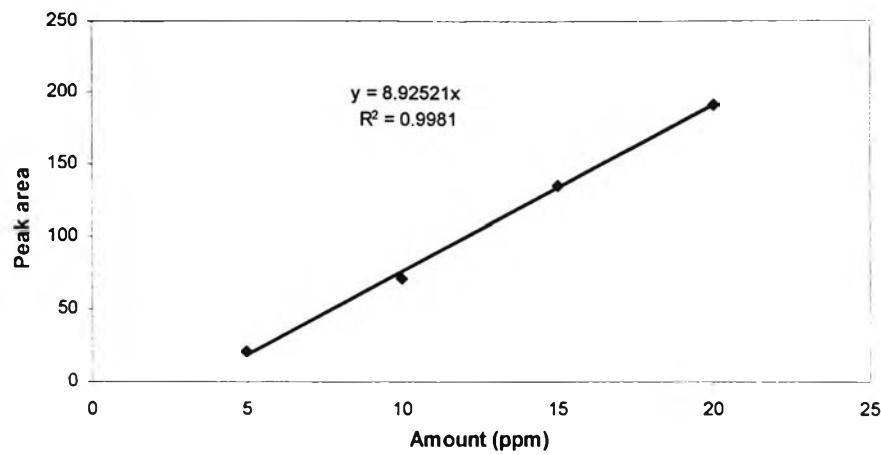
### C.3.4 Standard curve of vanillin

Retention time (min)	Amount (ppm)	Area	Name
12.669	5	62.0791	Vanillin
	10	193.06236	
	15	349.78372	
	20	498.93567	



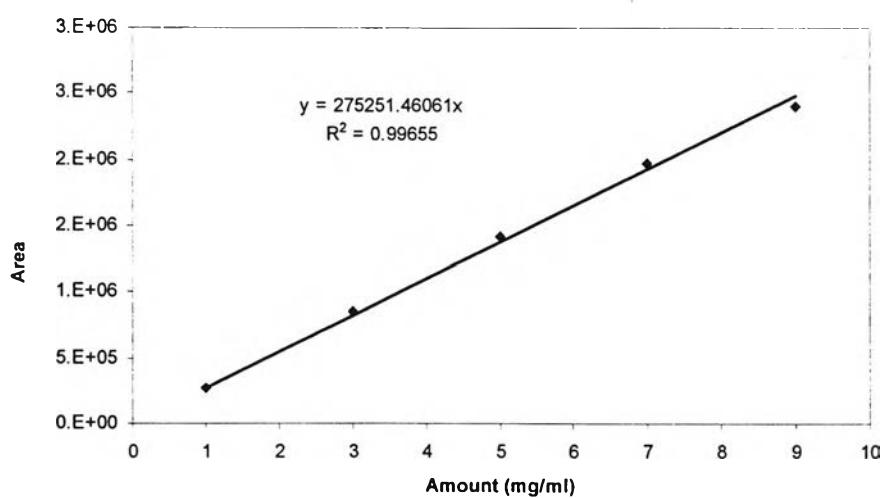
### C.3.5 Standard curve of syringaldehyde

Retention time (min)	Amount (ppm)	Area	Name
13.434	5	21.69074	Syringaldehyde
	10	71.85046	
	15	135.55247	
	20	191.68312	



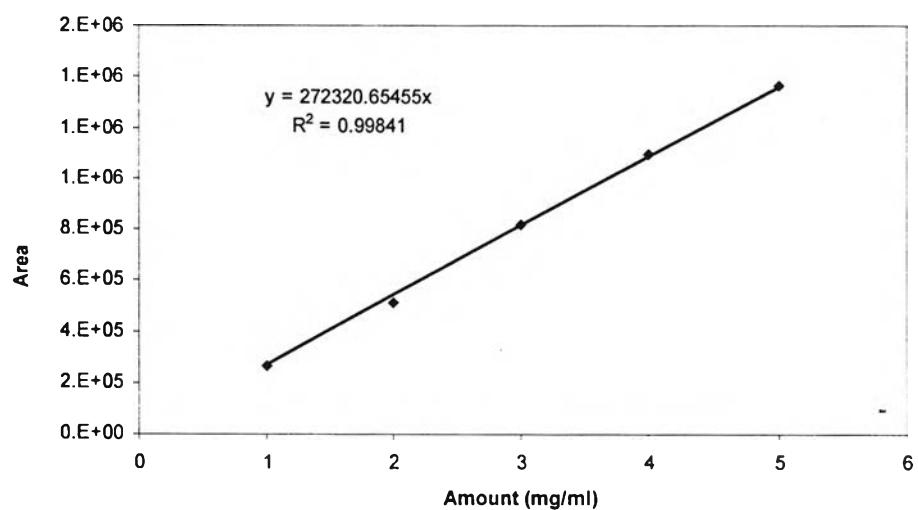
### C.3.6 Standard curve of glucose

Retention time (min)	Amount (mg/ml)	Area	Name
12.398	1	2.66802e5	Glucose
	3	8.56643e5	
	5	1.41855e6	
	7	1.97500e6	
	9	2.40689e6	



### C.3.7 Standard curve of xylose

Retention time (min)	Amount (mg/ml)	Area	Name
13.525	1	2.66839e5	Xylose
	2	5.11453e5	
	3	8.20077e5	
	4	1.09644e6	
	5	1.36838e6	



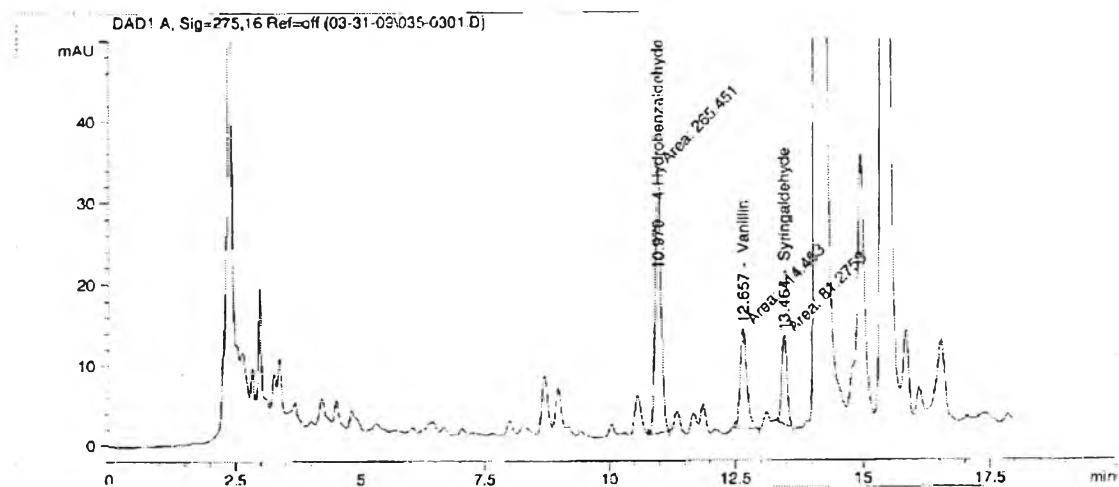
## APPENDIX D

### Sugar and byproducts in hydrolysate

#### D.1 Sugar and byproducts in Ca(OH)<sub>2</sub> pretreatment hydrolysate

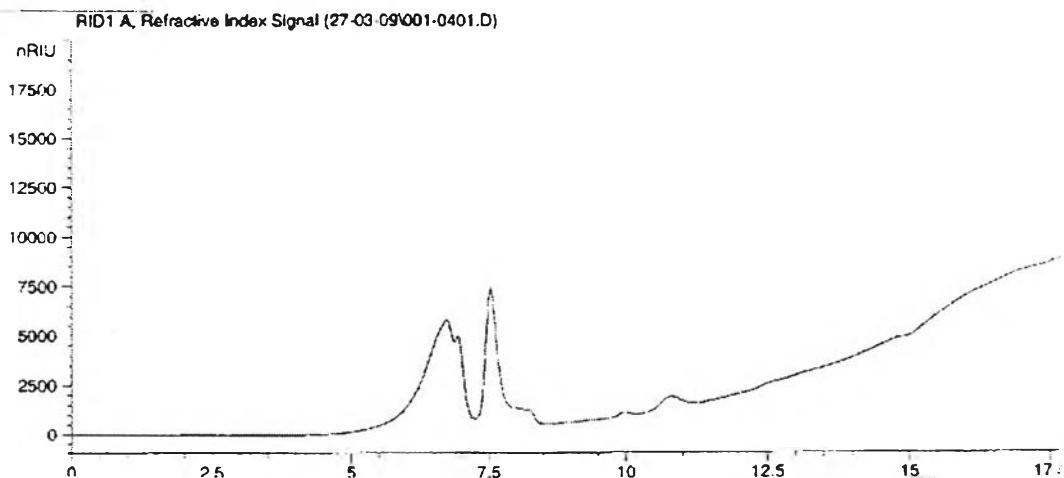
**Sample 1:** Reducing sugar 0.056 mg/ml

- Inhibitors



Retention time (min)	Area	Name	Amount (ppm)	Amount (mg/ml)
5.025	-	Hydroxymethyl furfural (HMF)	-	-
6.552	-	Furfural	-	-
10.979	265.45142	4-Hydrobenzaldehyde	7.29459e-1	0.00073
12.657	114.46322	Vanillin	5.74012e-1	0.00057
13.464	81.27534	Syringaldehyde	1.26415	0.00126

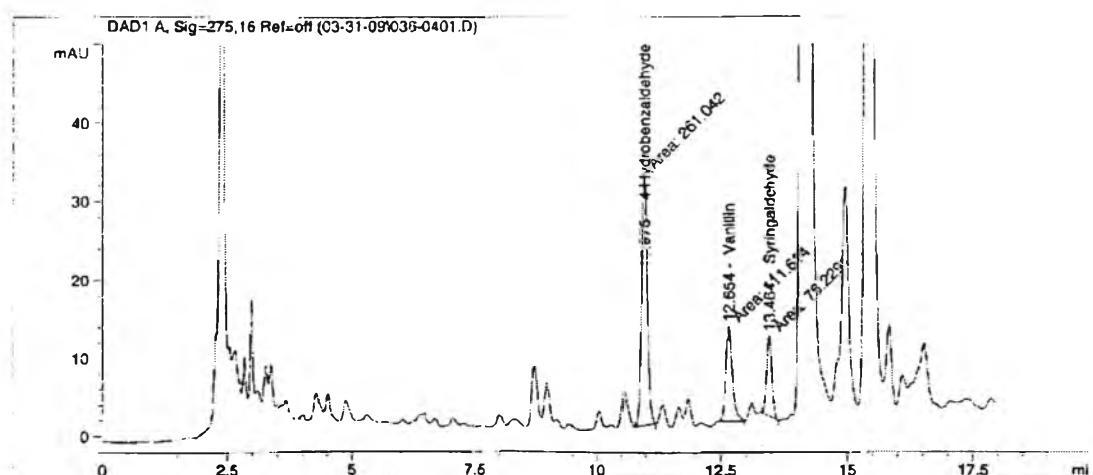
- Sugar



Retention time (min)	Area	Name	Amount (ng/ul)	Amount (mg/ml)
12.327	-	Glucose	-	-
13.479	-	Xylose	-	-

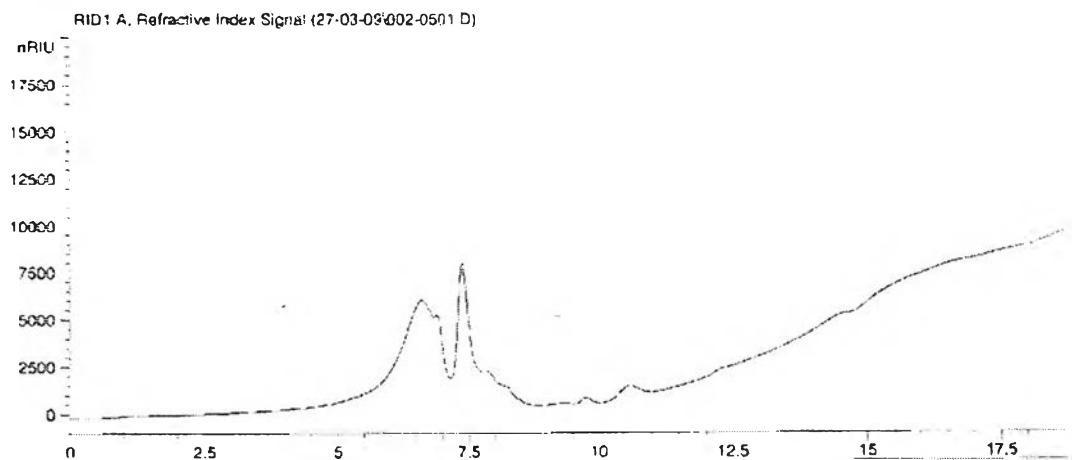
Sample 2: Reducing sugar 0.054 mg/ml

- Inhibitors



Retention time (min)	Area	Name	Amount (ppm)	Amount (mg/ml)
5.025	-	Hydroxymethyl furfural (HMF)	-	-
6.552	-	Furfural	-	-
10.975	261.04178	4-Hydrobenzaldehyde	7.17342e-1	0.000717
12.654	111.61407	Vanillin	5.59724e-1	0.000560
13.464	76.22906	Syringaldehyde	1.18566	0.001180

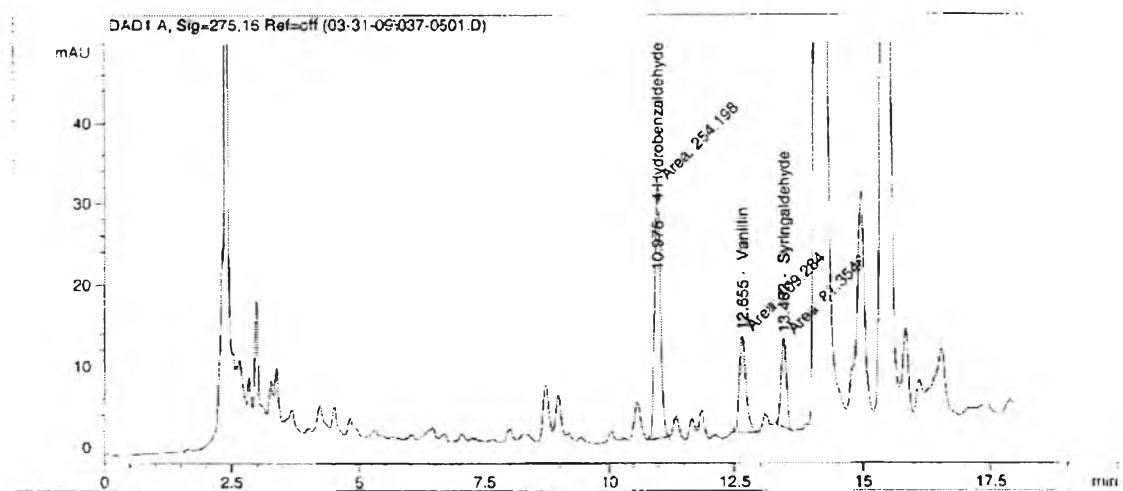
- Sugar



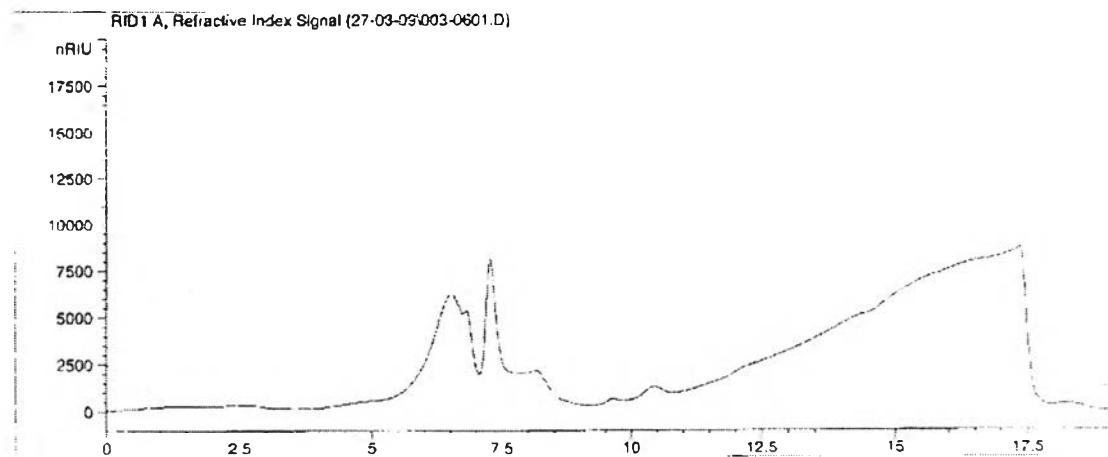
Retention time (min)	Area	Name	Amount (ng/uL)	Amount (mg/ml)
12.327	-	Glucose	-	-
13.479	-	Xylose	-	-

**Sample 3:** Reducing sugar 0.059 mg/ml

- Inhibitors



Retention time (min)	Area	Name	Amount (ppm)	Amount (mg/ml)
5.025	-	Hydroxymethyl furfural (HMF)	-	-
6.552	-	Furfural	-	-
10.975	254.19751	4-Hydrobenzaldehyde	6.98534e-1	0.000698
12.655	109.28412	Vanillin	5.48040e-1	0.000548
13.463	81.35482	Syringaldehyde	1.26538	0.001180

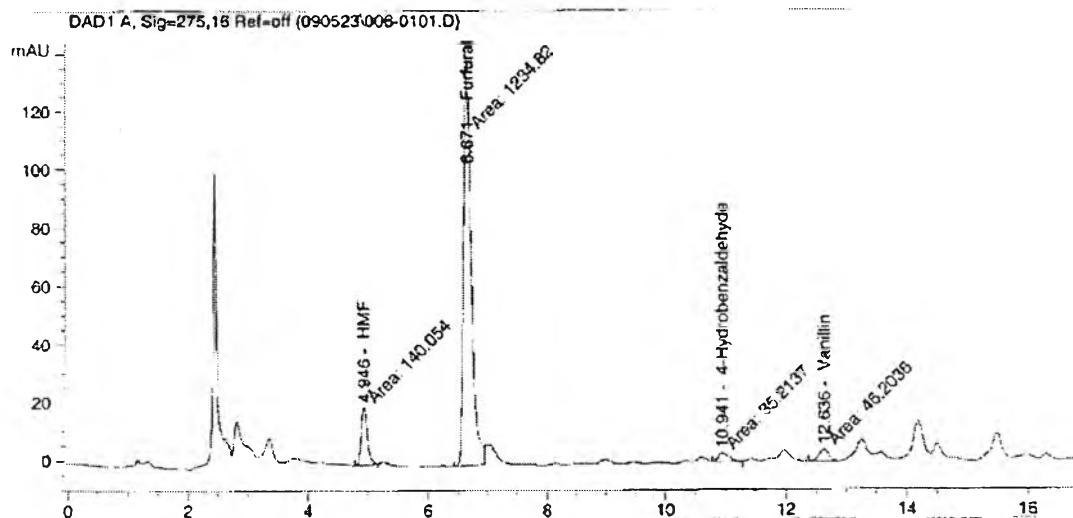
- Sugar

Retention time (min)	Area	Name	Amount (ng/uL)	Amount (mg/ml)
12.327	-	Glucose	-	-
13.479	-	Xylose	-	-

## D.2 Sugar and byproducts in H<sub>2</sub>S0<sub>4</sub> pretreatment hydrolysate

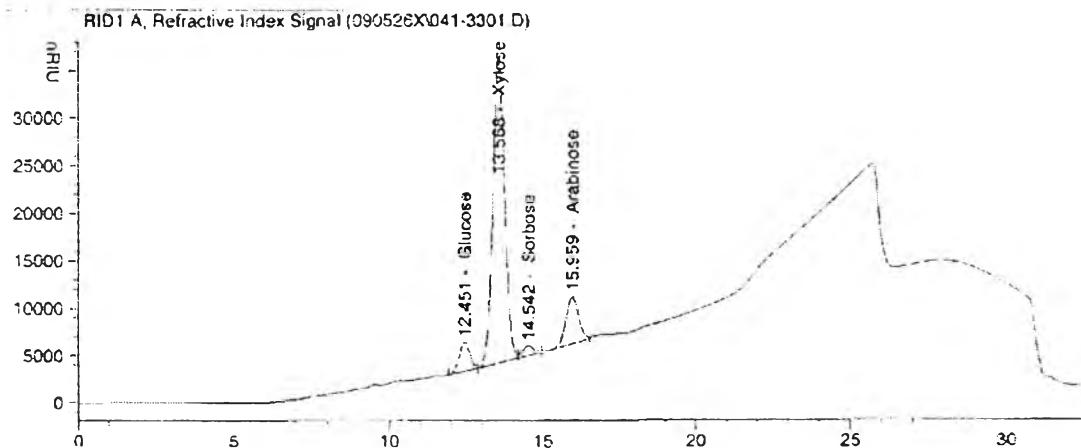
**Sample 1:** Reducing sugar 9.519 mg/ml

### - Inhibitors



Retention time (min)	Area	Name	Amount (ppm)	Amount (mg/ml)
4.946	140.05389	Hydroxymethyl furfural (HMF)	6.35864	0.00636
6.671	1234.81677	Furfural	19.99388	0.01999
10.941	35.21370	4-Hydrobenzaldehyde	1.36509	0.00137
12.636	46.20361	Vanillin	1.98365	0.001984
13.434	-	Syringaldehyde	-	-

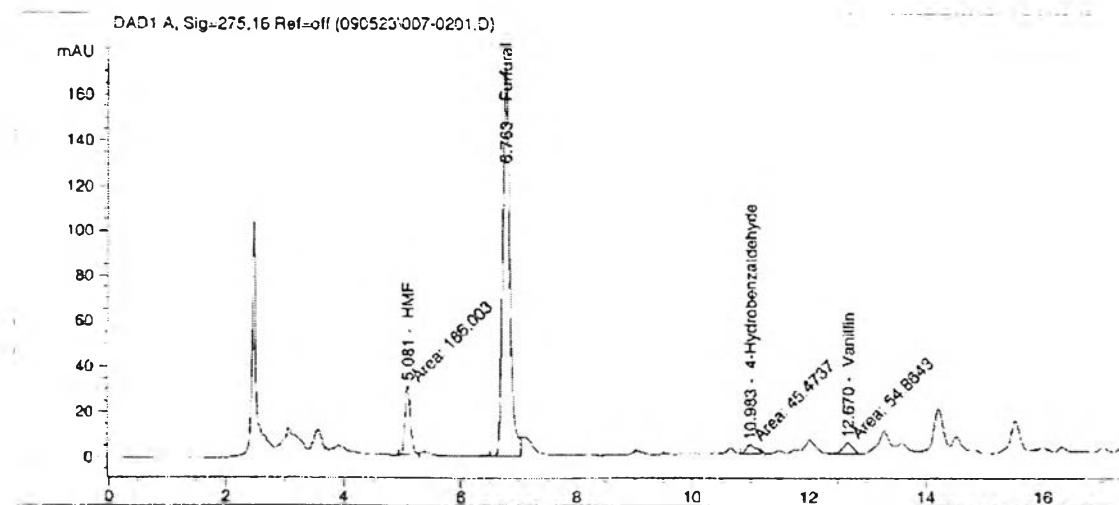
- Sugar



Retention time (min)	Area	Name	Amount (ng/ul)	Amount (mg/ml)
12.399	4.131e5	Glucose	1.501	1.501
13.568	8.580e5	Xylose	3.15071	3.15071

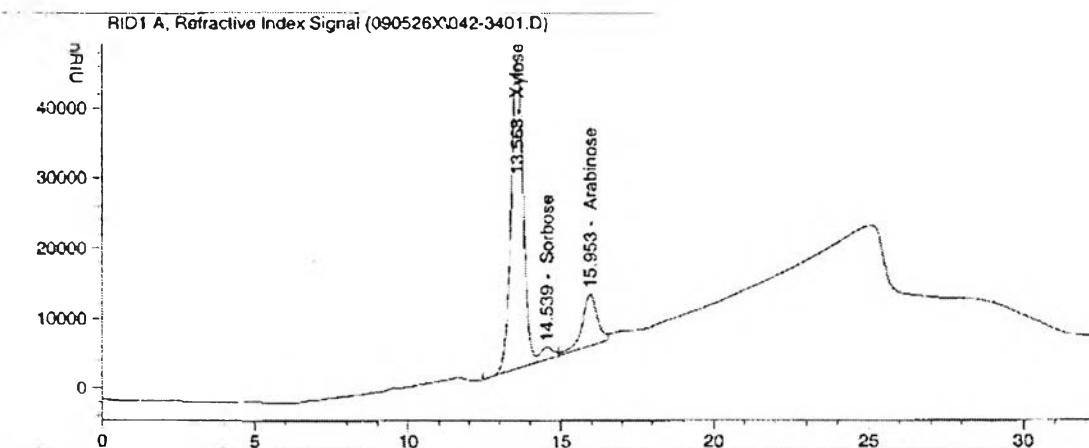
**Sample 2:** Reducing sugar 11.148 mg/ml

- Inhibitors



Retention time (min)	Area	Name	Amount (ppm)	Amount (mg/ml)
5.081	186.00275	Hydroxymethyl furfural (HMF)	8.44478	0.00844
6.763	1459.18420	Furfural	23.62679	0.02363
10.983	45.47371	4-Hydrobenzaldehyde	1.76283	0.00176
12.670	54.86431	Vanillin	2.35548	0.00235
13.434	-	Syringaldehyde	-	-

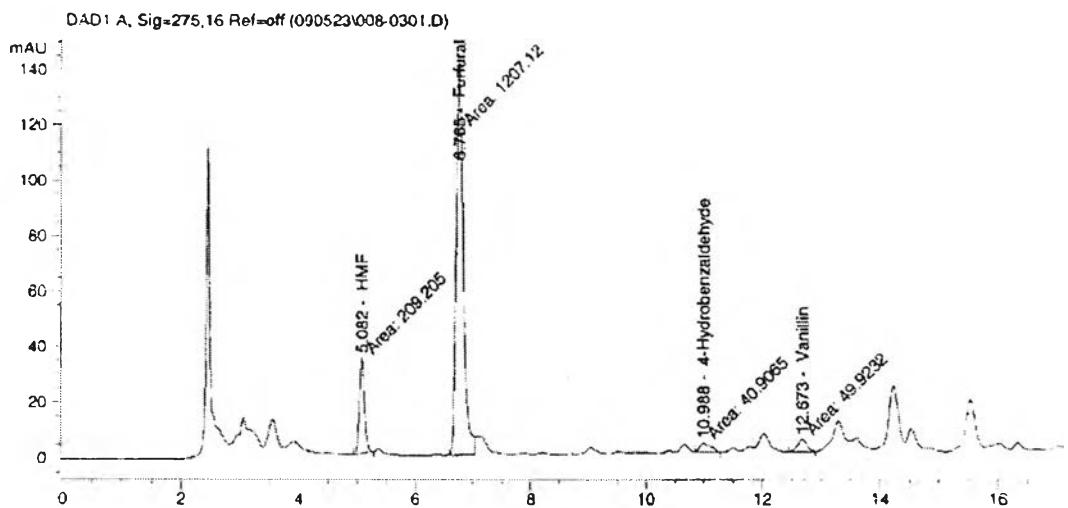
- Sugar



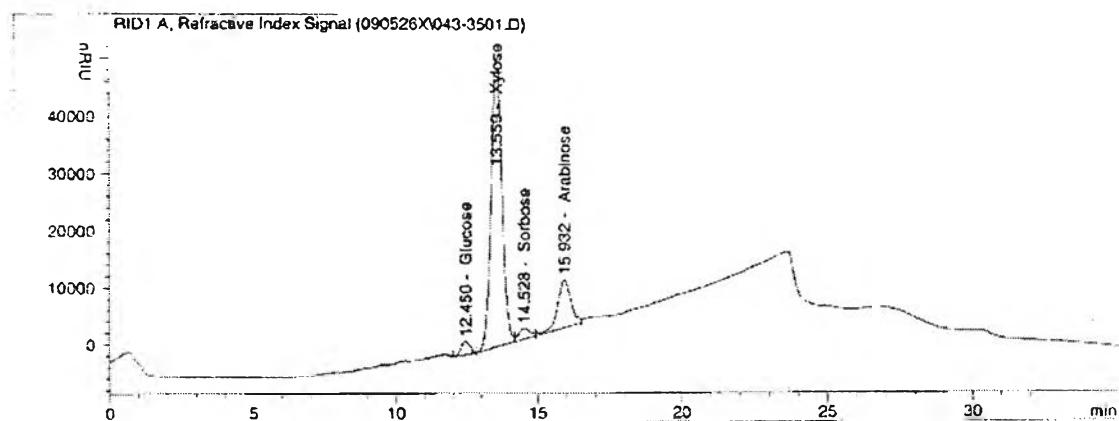
Retention time (min)	Area	Name	Amount (ng/uL)	Amount (mg/ml)
12.399	2.927e5	Glucose	1.063	1.063
13.568	1.196e6	Xylose	4.3919	3.15071

**Sample 3:** Reducing sugar 11.410 mg/ml

- Inhibitors



Retention time (min)	Area	Name	Amount (ppm)	Amount (mg/ml)
5.082	209.20476	Hydroxymethyl furfural (HMF)	9.49818	0.00950
6.765	1207.12183	Furfural	19.54545	0.01955
10.988	40.90649	4-Hydrobenzaldehyde	1.58577	0.00159
12.673	49.92317	Vanillin	2.14335	0.00214
13.434	-	Syringaldehyde	-	-

- Sugar

Retention time (min)	Area	Name	Amount (ng/uL)	Amount (mg/ml)
12.404	4.118e5	Glucose	1.496	1.496
13.559	1.344e6	Xylose	4.9354	3.15071

## BIOGRAPHY

Miss Suwaphat Ratsamee was born in August 31, 1984 in Phichit, Thailand. She graduated from Department of Microbiology, Faculty of Science, Chulalongkorn University, Thailand with Bachelor Degree of Science since 2007.

### Academic presentation :

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