

CHAPTER IV

RESULTS AND DISCUSSION

Absorption spectra scanning

Absorption spectra was used in preliminary study two simple redox reactions of ethanol which was oxidized by an acidic potassium permanganate (converted to colorless of manganese(II)) and an acidic potassium dichromate (converted to green color of chromium(III)). The experiment was performed and the absorption spectra were recorded by using UV-Visible spectrophotometer (Lambda 20, version 2.85.04 of UV Winlab software, Perkin Elmer, USA). Various solutions were consisted of: 1) ethanol (0, 1 and 5 %v/v) in acidic potassium permanganate solution (0.24 mmol/L KMnO_4 + 0.06 mol/L H_2SO_4) and 2) ethanol (0, 0.1, 0.5 and 1 %v/v) in acidic potassium dichromate solution (0.2 mol/L $\text{K}_2\text{Cr}_2\text{O}_7$ + 4.0 mol/L H_2SO_4). Conditions used of UV-Visible spectrophotometer are shown in Table 2.

Table 2 Conditions used of UV-Visible spectrophotometer

Parameters	Conditions used
Start wavelength	800 nm
End wavelength	400 nm
Scan speed	240 nm/min
Smooth	2 nm

For an acidic potassium permanganate used, it was found that the maximum wavelength was 525-550 nm (Figure 10). This wavelength was selected for ethanol determination because the absorption intensity of potassium permanganate decreased with addition of solution containing the increase ethanol concentration. From the results, a green-LED was selected as light source of FI-colorimetric system for ethanol determination by using an acidic potassium permanganate reagent solution because its emitting wavelength was approximate 525 nm. Under the light emitting principle of LED colorimetric detection, the positive-FI peaks were appeared by using green-LED

for detection. Peak height is proportional to the decrease in absorption intensity of potassium permanganate.

For an acidic potassium dichromate used, it was indicated that the maximum wavelength was 600 nm (Figure 11). This wavelength was selected for ethanol determination because the absorption intensity of chromium-(III) increased with addition of solution that increases in ethanol concentration. From the results, a red-LED was used as light source of FI-colorimetric system for ethanol determination by using acidic potassium dichromate reagent solution because its emitting wavelength was approximate 600 nm. Under the same principle, the negative-FI peaks were appeared by using red-LED for detection the increase absorption intensity of chromium-(III) product.

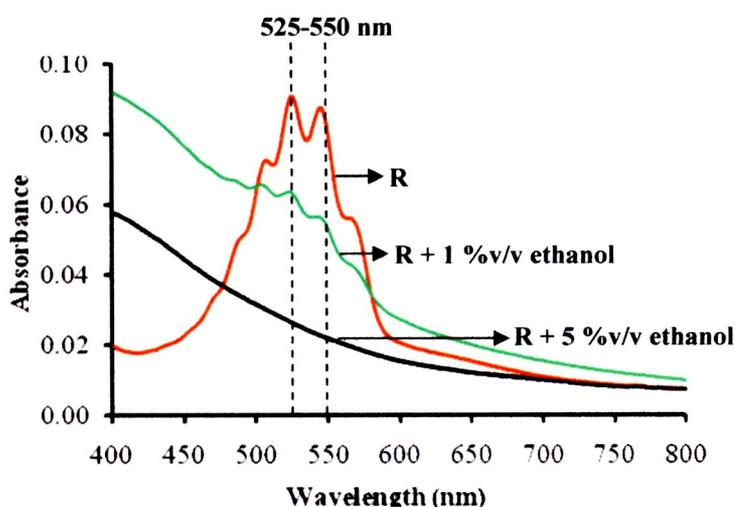


Figure 10 Absorption spectra of various standard ethanol solutions (0, 1 and 5 %v/v) in an acidic potassium permanganate reagent solution (R; 0.24 mmol/L KMnO_4 plus 0.06 mol/L H_2SO_4)

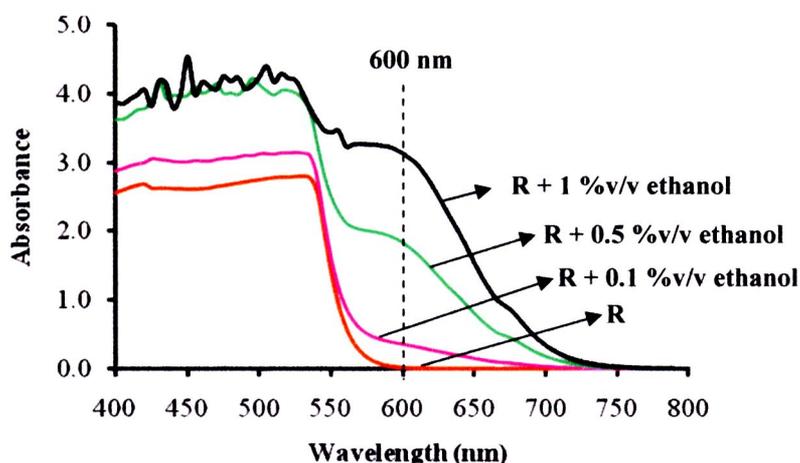


Figure 11 Absorption spectra of various standard ethanol solutions (0, 0.1, 0.5 and 1 %v/v) in an acidic potassium dichromate reagent solution (R; 0.2 mol/L $K_2Cr_2O_7$ plus 4 mol/L H_2SO_4)

Determination of ethanol in Thai white distilled liquor by FI-colorimetric system

1. Optimization of FI-colorimetric system by using an acidic potassium permanganate reagent solution

Preliminary conditions for ethanol determination were used as shown in Table 3. And optimum conditions study for the determination of ethanol by using an acidic potassium permanganate was as followed:

Table 3 Preliminary conditions used of FI-colorimetric system by using an acidic potassium permanganate reagent solution

Parameters	Used
Potassium permanganate (mmol/L)	0.2
Sulfuric acid (mol/L)	0.1
Flow rate (mL/min)	1.0
Reaction coil length (cm)	140
Sample volume (μ L)	90
Light source	green-LED

1.1 Effect of KMnO_4 concentrations

Potassium permanganate is an oxidizing agent to oxidize ethanol and its concentration affected to sensitivity and linearity of the calibration graph. Thus, the effect of KMnO_4 concentrations was studied. Using the manifold as shown in Figure 8 and preliminary conditions as shown in Table 3, blank and standard ethanol solutions (10, 20, 30 and 40 %v/v) were injected. Various concentration of KMnO_4 (0.1, 0.2, 0.3 and 0.4 mmol/L) were varied and optimized. The results are shown in Table 4 and Figure 12. It was found that all calibration graphs studied were linear in the range of 10-40 %v/v ethanol at KMnO_4 concentrations of 0.1-0.4 mol/L. The slope reached a plateau at 0.2-0.4 mmol/L of KMnO_4 . Below 0.1 mmol/L KMnO_4 , the decrease in peak height and slope was small due to the stoichiometric limitation of the reaction. A KMnO_4 concentration of 0.2 mmol/L was selected for further studies.

Table 4 Effect of KMnO_4 concentrations on peak height and slope of standard ethanol solutions

KMnO_4 (mmol/L)	Ethanol (%v/v)	Peak height (V)					Slope	R^2
		1	2	3	\bar{X}	SD		
0.1	0	0.00	0.00	0.00	0.00	0.00	0.0236	0.9803
	10	0.28	0.29	0.28	0.28	0.00		
	20	0.59	0.58	0.56	0.58	0.02		
	30	0.85	0.85	0.80	0.83	0.03		
	40	1.00	0.97	0.96	0.98	0.02		
0.2	0	0.00	0.00	0.00	0.00	0.00	0.0370	0.9963
	10	0.31	0.31	0.29	0.30	0.01		
	20	0.64	0.62	0.63	0.63	0.01		
	30	0.99	1.00	0.98	0.99	0.01		
	40	1.42	1.41	1.42	1.42	0.00		
0.3	0	0.00	0.00	0.00	0.00	0.00	0.0342	0.9861
	10	0.24	0.23	0.23	0.23	0.00		
	20	0.59	0.60	0.58	0.59	0.01		
	30	0.96	0.97	0.97	0.97	0.01		
	40	1.33	1.22	1.19	1.25	0.06		

Table 4 (Cont.)

KMnO ₄ (mmol/L)	Ethanol (%v/v)	Peak height (V)					Slope	R ²
		1	2	3	\bar{X}	SD		
	0	0.00	0.00	0.00	0.00	0.00		
	10	0.18	0.18	0.17	0.17	0.00		
0.4	20	0.50	0.46	0.46	0.48	0.02	0.0335	0.9962
	30	0.90	0.87	0.84	0.87	0.03		
	40	1.18	1.15	1.15	1.16	0.02		

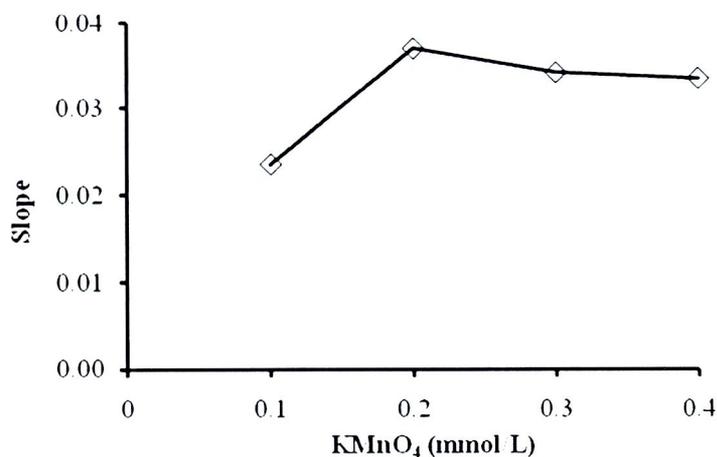


Figure 12 Effect of KMnO₄ concentrations on slope of standard ethanol solutions (10, 20, 30 and 40 %v/v)

1.2 Effect of H₂SO₄ concentrations

Sulfuric acid is a portion of the reaction and affected to sensitivity of ethanol determination. Thus, the effect of H₂SO₄ concentrations was studied. Various concentration of H₂SO₄ (0.05, 0.1, 0.2, 0.3 and 0.5 mol/L) were varied and optimized. The results are shown in Table 5 and Figure 13. It was found that no significant difference in peak heights and slope for both 10 and 40 %v/v ethanol over a H₂SO₄ concentration of 0.05-0.5 mol/L. A H₂SO₄ concentration of 0.25 mol/L was selected for further studies.

Table 5 Effect of H₂SO₄ concentrations on peak height and slope of standard ethanol solutions

H ₂ SO ₄ (mol/L)	Ethanol (%v/v)	Peak height (V)					Slope	R ²
		1	2	3	\bar{X}	SD		
0.05	0	0.00	0.00	0.00	0.00	0.00	0.0505	1.0000
	10	0.37	0.39	0.40	0.39	0.02		
	40	1.92	1.89	1.89	1.90	0.02		
0.1	0	0.00	0.00	0.00	0.00	0.00	0.0505	1.0000
	10	0.36	0.41	0.40	0.39	0.00		
	40	1.87	1.90	1.94	1.90	0.04		
0.2	0	0.00	0.00	0.00	0.00	0.00	0.0511	1.0000
	10	0.37	0.40	0.40	0.39	0.02		
	40	1.92	1.94	1.96	1.94	0.02		
0.3	0	0.00	0.00	0.00	0.00	0.00	0.0509	1.0000
	10	0.41	0.41	0.42	0.41	0.01		
	40	1.94	1.94	1.94	1.94	0.00		
0.5	0	0.00	0.00	0.00	0.00	0.00	0.0509	1.0000
	10	0.42	0.42	0.43	0.42	0.01		
	40	1.90	1.96	1.94	1.93	0.03		

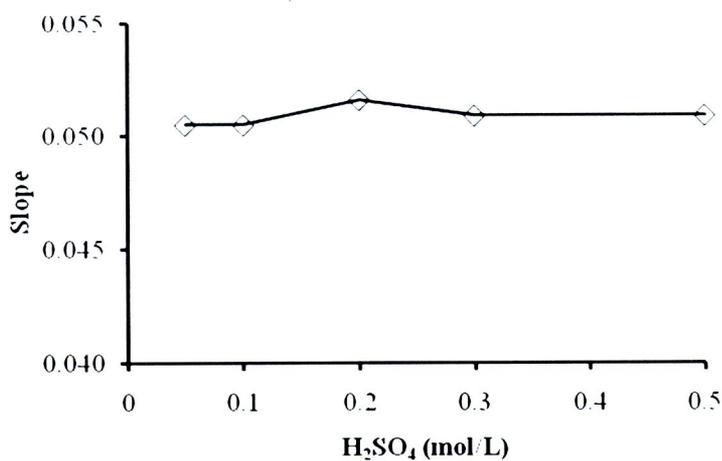


Figure 13 Effect of H₂SO₄ concentrations on peak height of standard ethanol solutions (10 and 40 %v/v)

1.3 Effect of flow rates of carrier and reagent streams

Flow rate of carrier and reagent streams is a key parameter of FIA system to control dispersion, which is effect to peak heights and slopes of ethanol determination. Thus, the effect of flow rates of carrier and reagent streams was studied. Various flow rates of two streams (0.6, 1.0, 1.4 and 1.8 mol/L) were varied and optimized. The results are shown in Table 6 and Figure 14. Over 1.0 mL/min of flow rate of both streams, peak heights and slope were rapidly decreased because of low dispersion occurred in the system. Below 1.0 mL/min, elevated dispersion and lower sample rate were arisen. Thus, the flow rate of 1.0 mL/min was chosen as giving the highest peak height and slope.

Table 6 Effect of flow rates of carrier and reagent streams on peak height and slope of standard ethanol solutions

Flow rate (mL/min)	Ethanol (%v/v)	Peak height (V)					Slope	R ²
		1	2	3	\bar{X}	SD		
0.6	0	0.00	0.00	0.00	0.00	0.00	0.0377	0.9874
	10	0.56	0.55	0.54	0.55	0.01		
	20	1.04	1.02	1.01	1.02	0.02		
	30	1.44	1.40	1.36	1.40	0.04		
	40	1.69	1.68	1.67	1.68	0.01		
1.0	0	0.00	0.00	0.00	0.00	0.00	0.0467	0.9931
	10	0.27	0.27	0.31	0.28	0.02		
	20	0.85	0.81	0.87	0.84	0.03		
	30	1.31	1.29	1.29	1.30	0.01		
	40	1.69	1.69	1.67	1.69	0.01		
1.4	0	0.00	0.00	0.00	0.00	0.00	0.0318	0.9939
	10	0.26	0.30	0.29	0.28	0.02		
	20	0.60	0.59	0.58	0.59	0.01		
	30	0.86	0.84	0.88	0.86	0.02		
	40	1.26	1.22	1.27	1.25	0.03		

Table 6 (Cont.)

Flow rate (mL/min)	Ethanol (%v/v)	Peak height (V)					Slope	R ²
		1	2	3	\bar{X}	SD		
1.8	0	0.00	0.00	0.00	0.00	0.00	0.0200	0.9907
	10	0.22	0.22	0.22	0.22	0.00		
	20	0.47	0.45	0.49	0.47	0.02		
	30	0.84	0.83	0.86	0.84	0.01		
	40	0.63	0.60	0.61	0.61	0.00		

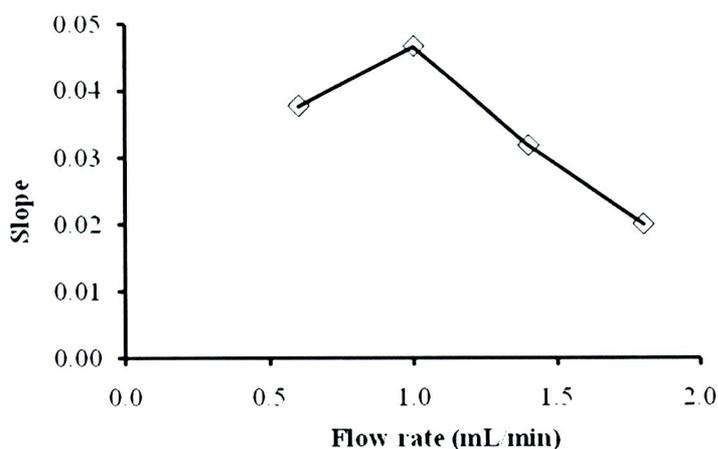


Figure 14 Effect of flow rates of carrier and reagent streams on slope of standard ethanol solutions (10, 20, 30 and 40 %v/v)

1.4 Effect of reaction coil lengths

In order to achieve good mixing of sample and reagent solutions and high sensitivity, the effect of reaction coil length (teflon tubing, 0.89 mm i.d.) was studied. Various reaction coil lengths (100, 140 and 210 cm) were varied and optimized. The results are shown in Table 7 and Figure 15. The results indicate that a reaction coil length of 140 cm was chosen as giving the highest peak height and slope.

Table 7 Effect of reaction coil lengths on peak height and slope of standard ethanol solutions

Reaction coil (cm)	Ethanol (%v/v)	Peak height (V)					Slope	R ²
		1	2	3	\bar{X}	SD		
100	0	0.00	0.00	0.00	0.00	0.00	0.0398	0.9946
	10	0.34	0.36	0.37	0.36	0.01		
	20	0.74	0.70	0.72	0.72	0.02		
	30	1.13	1.02	1.09	1.08	0.05		
	40	1.55	1.58	1.56	1.56	0.02		
140	0	0.00	0.00	0.00	0.00	0.00	0.0415	0.9996
	10	0.26	0.27	0.26	0.26	0.01		
	20	0.68	0.68	0.68	0.68	0.00		
	30	1.11	1.12	1.11	1.11	0.00		
	40	1.48	1.48	1.55	1.50	0.04		
210	0	0.00	0.00	0.00	0.00	0.00	0.0353	0.9998
	10	0.25	0.26	0.26	0.26	0.01		
	20	0.62	0.63	0.62	0.62	0.00		
	30	0.97	0.97	0.94	0.96	0.02		
	40	1.30	1.32	1.34	1.32	0.02		

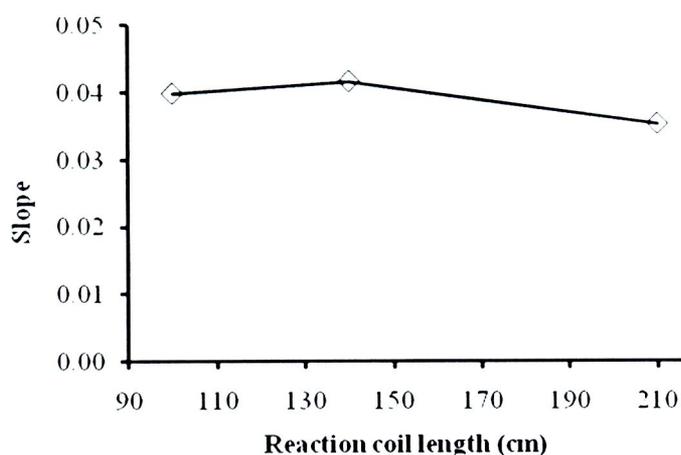


Figure 15 Effect of reaction coil lengths on slope of standard ethanol solutions (10, 20, 30 and 40 %v/v)



1.5 Effect of sample volumes

The effect of sample volume was studied because of effective dispersion and sensitivity of the system. Using conditions described in 1.4, blank and standard ethanol solutions (10, 20, 30 and 40 %v/v) were injected. Various sample volumes (90, 120, 150 and 180 μL) were varied and optimized. The results are shown in Table 8 and Figure 16. The results indicate that the increase in sample volume decreased peak heights and slopes and increased dispersion. A sample volume of 90 μL was chosen.

Table 8 Effect of sample volumes on peak height and slope of standard ethanol solutions

Sample volume (μL)	Ethanol (%v/v)	Peak height (V)					Slope	R^2
		1	2	3	\bar{X}	SD		
90	0	0.00	0.00	0.00	0.00	0.00	0.0503	0.9945
	10	0.26	0.26	0.27	0.26	0.00		
	20	0.72	0.69	0.69	0.70	0.02		
	30	1.18	1.17	1.20	1.18	0.01		
	40	1.81	1.78	1.76	1.78	0.02		
120	0	0.00	0.00	0.00	0.00	0.00	0.0473	0.9610
	10	0.32	0.30	0.29	0.30	0.02		
	20	0.87	0.85	0.80	0.84	0.03		
	30	1.50	1.50	1.45	1.48	0.03		
	40	1.68	1.66	1.67	1.67	0.01		
150	0	0.00	0.00	0.00	0.00	0.00	0.0428	0.9100
	10	0.36	0.41	0.39	0.38	0.03		
	20	1.05	1.00	0.98	1.01	0.03		
	30	1.52	1.59	1.59	1.57	0.04		
	40	1.64	1.62	1.62	1.62	0.01		
180	0	0.00	0.00	0.00	0.00	0.00	0.0356	0.7509
	10	0.45	0.46	0.43	0.45	0.02		
	20	1.33	1.32	1.34	1.33	0.01		
	30	1.58	1.56	1.56	1.57	0.01		
	40	1.57	1.55	1.54	1.55	0.01		

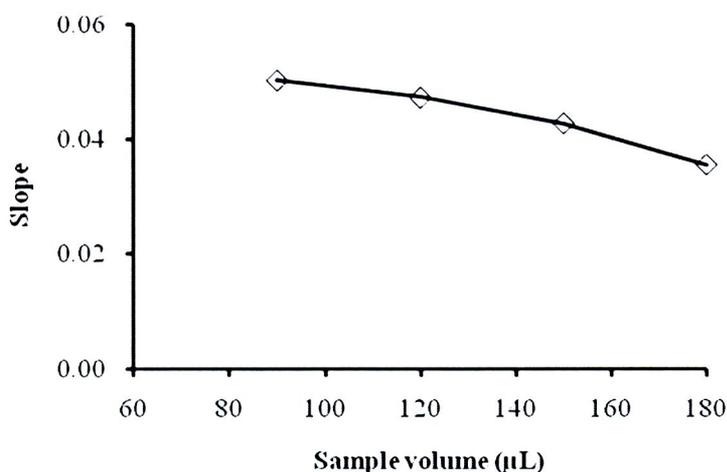


Figure 16 Effect of sample volumes on slope of standard ethanol solutions (10, 20, 30 and 40 %v/v)

1.6 Stability study of reagent solution

Because of the instability of an acidic potassium permanganate reagent solution, the stability of this reagent (0.2 mmol/L KMnO_4 in 0.25 mol/L H_2SO_4) was studied from 0 to 480 min by using UV-Visible spectrophotometer (Lamda 20, version 2.85.04 of UV Winlab software, Perkin Elmer, USA). The results are shown in Table 9 and Figure 17. The results indicate that this reagent could be used and operated within three hours (180 min) and should be freshly prepared.

Table 9 Stability study of reagent solution

Time (min)	Absorbance	Time (min)	Absorbance
0	0.4152	180	0.4122
10	0.4147	195	0.4128
30	0.4149	210	0.4117
40	0.4150	225	0.4114
50	0.4150	240	0.4112
60	0.4136	260	0.4106
75	0.4142	280	0.4103
90	0.4129	300	0.4096
105	0.4127	320	0.4092

Table 9 (Cont.)

Time (min)	Absorbance	Time (min)	Absorbance
120	0.4133	340	0.4083
135	0.4131	360	0.4082
150	0.4130	420	0.4056
165	0.4128	480	0.4041

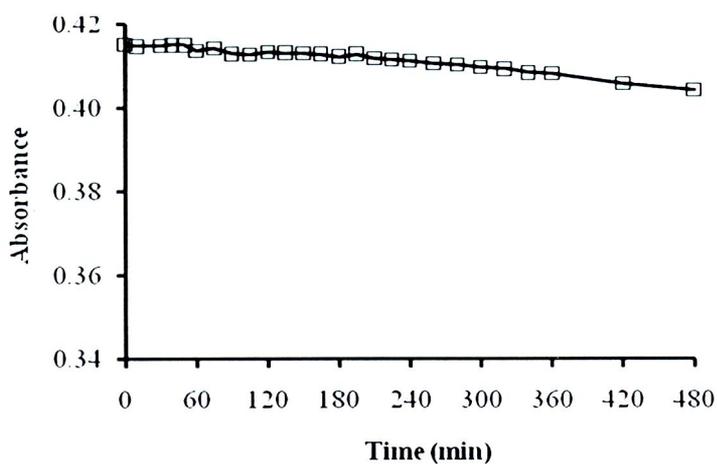


Figure 17 Stability study of reagent solution (0.2 mmol/L KMnO_4 in 0.25 mol/L H_2SO_4) by using UV-Visible spectrophotometer

1.7 Summary of condition used

The recommended FI-colorimetric manifold is depicted in Figure 8 and the optimum conditions are summarized in Table 10.

Table 10 Conditions used for the determination of ethanol

Parameters	Conditions Used
Potassium permanganate (mmol/L)	0.2
Sulfuric acid (mol/L)	0.25
Flow rate (mL/min)	1.0
Reaction coil length (cm)	140
Sample volume (μL)	90
Light source	green-LED

1.8 Calibration graph and detection limit

Using the manifold as shown in Figure 8 and the optimum conditions described in 1.7, blank and standard ethanol solutions (10, 20, 30 and 40 %v/v) were injected. A calibration graph was plotted between peak heights obtained versus standard ethanol concentration. A detection limit was calculated using Miller & Miller method (described in Appendix B.1). The results are shown in Table 11, Figure 18 and Figure 19. Linearity was obtained in the range of 10-40 %v/v ethanol. The detection limit was 1.2 %v/v ethanol. The relative standard deviation (RSD) was in the range of 1.7-3.7 % and the sample throughput was 72 injections per hour.

Table 11 Calibration data for ethanol determination by using acidic KMnO_4 reagent solution

Ethanol (%v/v)	Peak height (V)					
	1	2	3	\bar{X}	SD	%RSD
10	0.35	0.33	0.33	0.34	0.01	2.9
20	0.85	0.81	0.82	0.83	0.02	2.4
30	1.30	1.31	1.39	1.35	0.05	3.7
40	1.83	1.80	1.78	1.81	0.03	1.7

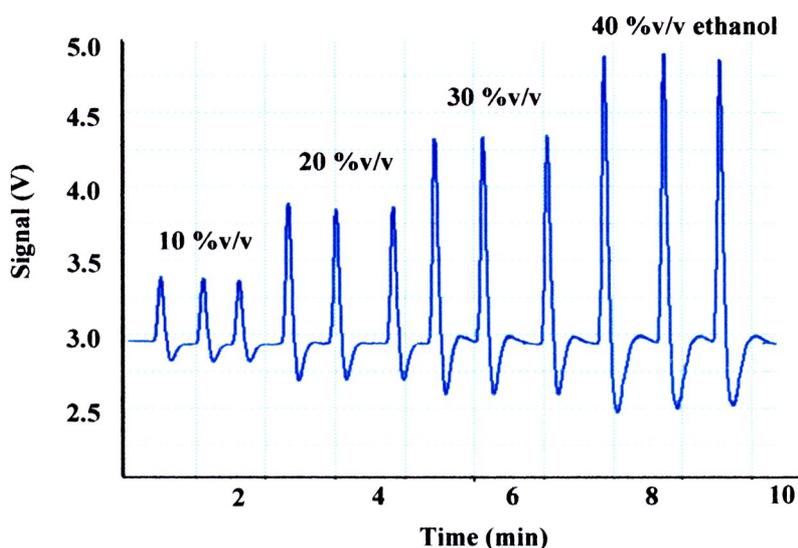


Figure 18 FI signals for the determination of ethanol by using an acidic KMnO_4 reagent solution

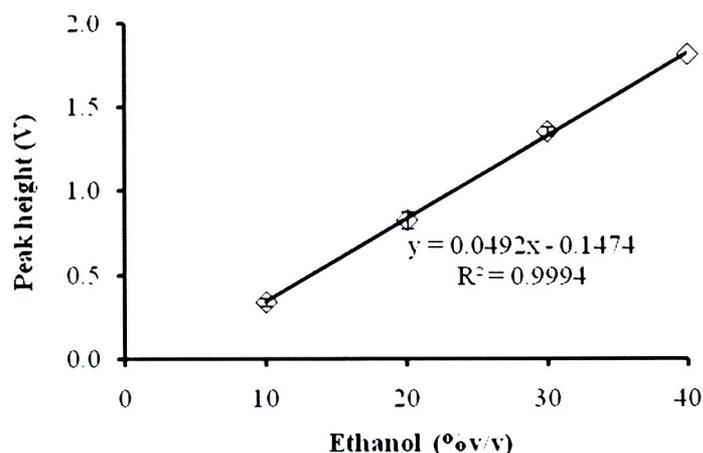


Figure 19 Calibration graph for the determination of ethanol (n=3) by using acidic KMnO_4

2. Optimization of the FI-colorimetric system by using an acidic potassium dichromate reagent solution

Preliminary conditions for ethanol determination were used as shown in Table 12. And optimum conditions study for the determination of ethanol by using an acidic potassium dichromate was as followed:

Table 12 Preliminary conditions used of FI-colorimetric system by using an acidic potassium dichromate reagent solution

Parameters	Used
Potassium dichromate (mol/L)	0.2
Sulfuric acid (mol/L)	4.0
Flow rate (ml/min)	1.0
Reaction coil length (cm)	140
Sample volume (μL)	90
Light source	red-LED

2.1 Effect of $K_2Cr_2O_7$ Concentrations

Potassium dichromate is an oxidizing agent to oxidize ethanol and affected to sensitivity and linear range of calibration graph. Therefore, the effect of $K_2Cr_2O_7$ concentrations was studied. Using the manifold as shown in Figure 8, blank and standard ethanol solutions (2 to 30 %v/v) were injected. Various concentrations of $K_2Cr_2O_7$ (0.05, 0.10, 0.20 and 0.30 mol/L) were varied and optimized. The results are shown in Table 13 and Figure 20. The results indicate that the difference in the linear range of calibration graph was occurred when $K_2Cr_2O_7$ was differed in concentration. The increase $K_2Cr_2O_7$ concentration decreased the linear range of calibration graph through its concentration increased the peak height and slope. Moreover, high $K_2Cr_2O_7$ concentration over 0.15 mol/L was difficultly dissolve in water. To compromise between sensitivity, linear range of calibration graph, dissolubility, the $K_2Cr_2O_7$ concentration of 0.15 mol/L was selected for further studies.

Table 13 Effect of $K_2Cr_2O_7$ concentrations on peak height and slope of standard ethanol solutions

$K_2Cr_2O_7$ (mol/L)	Ethanol (%v/v)	Peak height (V)					Slope	R^2
		1	2	3	\bar{X}	SD		
0.05	0	0.00	0.00	0.00	0.00	0.00	0.1649	0.9968
	10	0.46	0.46	0.45	0.46	0.01		
	20	2.25	2.27	2.28	2.27	0.02		
	30	3.79	3.77	3.70	3.75	0.05		
0.10	0	0.00	0.00	0.00	0.00	0.00	0.4313	0.9993
	5	0.72	0.73	0.65	0.70	0.05		
	6	1.16	1.18	1.14	1.16	0.02		
	8	1.99	1.97	2.04	2.00	0.03		
0.15	0	0.00	0.00	0.00	0.00	0.00	0.6977	0.9933
	4	0.69	0.69	0.68	0.69	0.01		
	5	1.46	1.46	1.45	1.46	0.01		
	6	2.42	2.41	2.35	2.39	0.04		
	7	2.66	2.81	2.72	2.73	0.08		
	8	3.59	3.63	3.52	3.58	0.06		
	10	4.91	4.91	4.99	4.94	0.05		

Table 13 (Cont.)

$K_2Cr_2O_7$ (mol/L)	Ethanol (%v/v)	Peak height (V)					Slope	R^2
		1	2	3	\bar{X}	SD		
0.20	0	0.00	0.00	0.00	0.00	0.00	1.3670	0.9834
	2	0.34	0.34	0.35	0.35	0.01		
	4	3.46	3.49	3.53	3.49	0.03		
	5	4.28	4.38	4.37	4.34	0.05		
0.30	0	0.00	0.00	0.00	0.00	0.00	1.4404	1.000
	2	1.41	1.42	1.37	1.40	0.03		
	4	4.29	4.28	4.27	4.28	0.01		
	0	0.00	0.00	0.00	0.00	0.00		

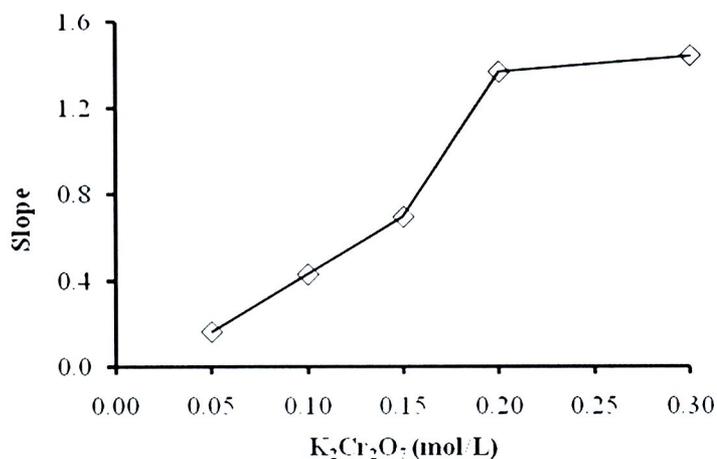


Figure 20 Effect of $K_2Cr_2O_7$ concentrations on slope of standard ethanol solutions, with different ranges of ethanol concentration during 0-30 %v/v

2.2 Effect of H_2SO_4 Concentrations

Sulfuric acid is a portion of the reaction that affected to sensitivity and calibration graph of ethanol determination. Thus, the effect of H_2SO_4 concentrations was studied. Using conditions described in 2.1, blank and standard ethanol solutions (4 to 40 %v/v) were injected. Various concentrations of H_2SO_4 (1.0, 2.0, 3.0 and 4.0 mol/L) were varied and optimized. The results are shown in Table 14

and Figure 21. The results indicate that difference in the linear range of calibration graph was arised when H₂SO₄ concentration was differed. The increase H₂SO₄ concentration decreased the linear range of calibration graph through its concentration increased the peak height and slope. Moreover, high H₂SO₄ concentration produced reagent bubble. To compromise between sensitivity, linear range of calibration graph, less reagent bubbles and expense of chemicals, the H₂SO₄ concentration of 4.0 mol/L was selected for further studies.

Table 14 Effect of H₂SO₄ concentrations on peak height and slope of standard ethanol solutions

H ₂ SO ₄ (mol/L)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
1.0	0	0.06	0.06	0.06	0.06	0.00	0.00	0.0012	0.9624
	2	0.06	0.06	0.06	0.06	0.00	0.00		
	4	0.06	0.06	0.06	0.06	0.00	0.00		
	6	0.06	0.06	0.06	0.06	0.00	0.00		
	8	0.06	0.06	0.06	0.06	0.00	0.00		
	10	0.08	0.08	0.08	0.08	0.02	0.00		
	20	0.08	0.08	0.08	0.08	0.02	0.00		
	30	0.09	0.10	0.09	0.09	0.03	0.00		
40	0.10	0.10	0.10	0.10	0.04	0.00			
2.0	0	0.08	0.08	0.08	0.08	0.00	0.00	0.003	0.9949
	2	0.08	0.08	0.08	0.08	0.00	0.00		
	4	0.09	0.09	0.09	0.09	0.02	0.00		
	6	0.10	0.10	0.10	0.10	0.02	0.00		
	8	0.11	0.11	0.11	0.11	0.03	0.00		
	10	0.12	0.12	0.11	0.12	0.04	0.00		
	20	0.14	0.14	0.14	0.14	0.06	0.00		
	30	0.17	0.17	0.18	0.17	0.10	0.00		
40	0.20	0.20	0.20	0.20	0.12	0.00			



Table 14 (Cont.)

H ₂ SO ₄ (mol/L)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
3.0	0	0.09	0.09	0.09	0.09	0.00	0.00	0.005	0.9995
	2	0.10	0.10	0.10	0.10	0.01	0.00		
	4	0.11	0.11	0.11	0.11	0.02	0.00		
	6	0.13	0.13	0.13	0.13	0.04	0.00		
	8	0.15	0.14	0.15	0.15	0.06	0.00		
	10	0.18	0.18	0.18	0.18	0.09	0.00		
	20	0.21	0.21	0.21	0.21	0.12	0.00		
	30	0.26	0.25	0.26	0.26	0.17	0.00		
	40	0.31	0.31	0.31	0.31	0.22	0.00		
4.0	0	0.10	0.10	0.10	0.10	0.00	0.00	0.0106	0.9991
	2	0.12	0.12	0.12	0.12	0.02	0.00		
	4	0.13	0.13	0.13	0.13	0.03	0.00		
	6	0.15	0.15	0.15	0.15	0.05	0.00		
	8	0.20	0.19	0.19	0.19	0.09	0.01		
	10	0.20	0.21	0.20	0.20	0.10	0.01		
	20	0.33	0.33	0.34	0.33	0.23	0.00		
	30	0.43	0.45	0.43	0.43	0.33	0.01		
	40	0.53	0.56	0.55	0.55	0.44	0.02		

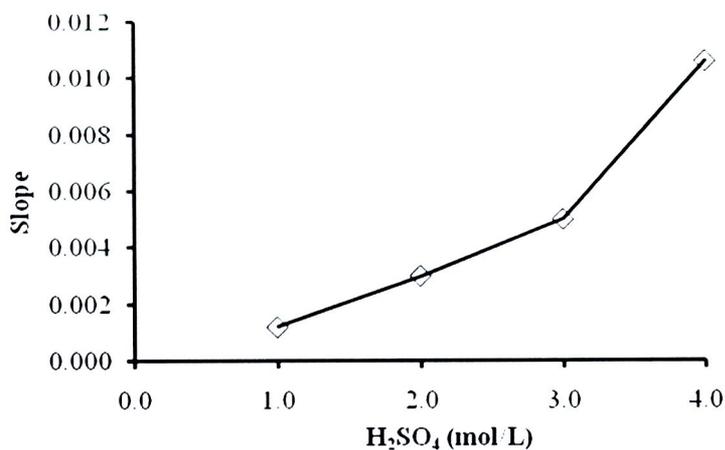


Figure 21 Effect of H₂SO₄ concentrations on slope of standard ethanol solutions, with different ranges of ethanol concentration during 0-40 %v/v

2.3 Effect of reaction coil lengths

In order to achieve good mixing of sample and reagent solution and high sensitivity, the effect of reaction coil length (teflon tubing, 0.89 mm i.d.) was studied. Using conditions described in 2.2, blank and standard ethanol solutions (2 to 10 %v/v) were injected. Various reaction coil lengths (100, 140 and 200 cm) were varied and optimized. The results are shown in Table 15 and Figure 22. The results indicate that a reaction coil length of 140 cm was chosen as giving the highest peak height and slope.

Table 15 Effect of reaction coil lengths on peak height and slope of standard ethanol solutions

Reaction coil length (cm)	Ethanol (%v/v)	Peak height (V)					Slope	R ²
		1	2	3	\bar{X}	SD		
100	0	0.00	0.00	0.00	0.00	0.00	0.4284	0.9744
	6	0.70	0.63	0.70	0.67	0.04		
	7	0.94	1.03	1.05	1.01	0.06		
	8	1.63	1.85	1.64	1.70	0.12		
	10	2.27	2.42	2.32	2.34	0.07		
140	0	0.00	0.00	0.00	0.00	0.00	0.7292	0.9928
	2	0.58	0.57	0.58	0.57	0.01		
	4	2.62	2.65	2.62	2.63	0.02		
	5	2.96	2.98	2.90	2.95	0.04		
	6	3.70	3.63	3.66	3.66	0.03		
	7	4.11	4.16	4.13	4.13	0.03		
200	0	0.00	0.00	0.00	0.00	0.00	0.6895	0.9975
	2	0.09	0.09	0.09	0.09	0.00		
	4	1.47	1.48	1.40	1.45	0.04		
	5	2.01	2.01	2.09	2.03	0.05		
	6	2.77	2.78	2.81	2.79	0.02		
	7	3.38	3.47	3.38	3.41	0.05		
	8	4.25	4.33	4.30	4.29	0.04		

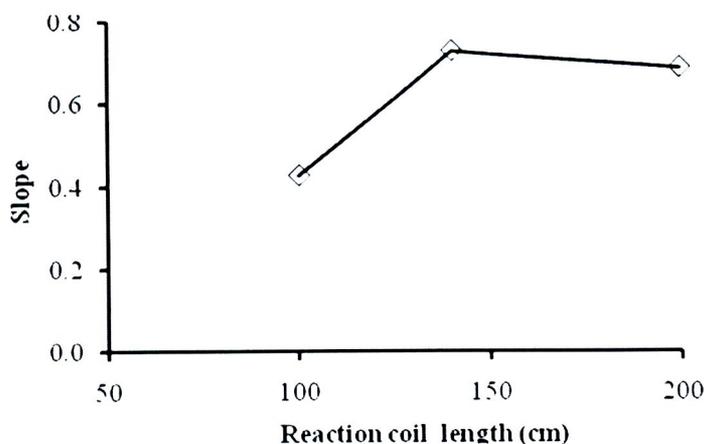


Figure 22 Effect of reaction coil lengths on slope of standard ethanol solutions, with different ranges of ethanol concentrations during 2-10 %v/v

2.4 Summary of conditions used

The recommended FI-colorimetric manifold is Figure 8 and the optimum conditions are summarized in Table 16.

Table 16 Conditions used for the determination of ethanol

Parameters	Conditions Used
Potassium dichromate (mol/L)	0.15
Sulfuric acid (mol/L)	4.0
Flow rate (mL/min)	1.0
Reaction coil length (cm)	140
Sample volume (μ L)	90
Light source	red-LED

2.5 Calibration graph and detection limit

Using the manifold as shown in Figure 8 and the optimum condition described in 2.4, blank and standard ethanol solutions (4, 5, 6 and 8 %v/v) were injected. A calibration graph was plotted between peak heights obtained versus standard ethanol concentration. A detection limit was calculated using Miller & Miller method (described in Appendix B.1). The results are shown in Table 17, Figure 23 and

Figure 24. Linearity was obtained in the range of 4-8 %v/v of ethanol. The detection limit was 0.3 %v/v ethanol. The RSD was in the range of 1.4-2.5 % and the sample throughput was 80 injections per hour.

Table 17 Calibration data for ethanol determination by using an acidic $K_2Cr_2O_7$ reagent solution

Ethanol (%v/v)	Peak height (V)					
	1	2	3	\bar{X}	SD	%RSD
4	1.90	1.99	1.93	1.94	0.04	2.1
5	2.79	2.84	2.69	2.77	0.07	2.5
6	3.59	3.52	3.50	3.54	0.05	1.4
8	4.93	4.98	4.82	4.91	0.08	1.6

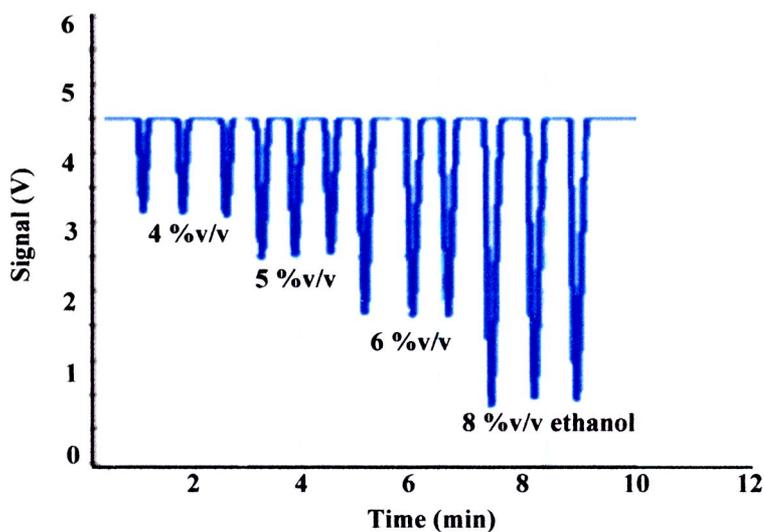


Figure 23 FI signals for the determination of ethanol by using acidic $K_2Cr_2O_7$

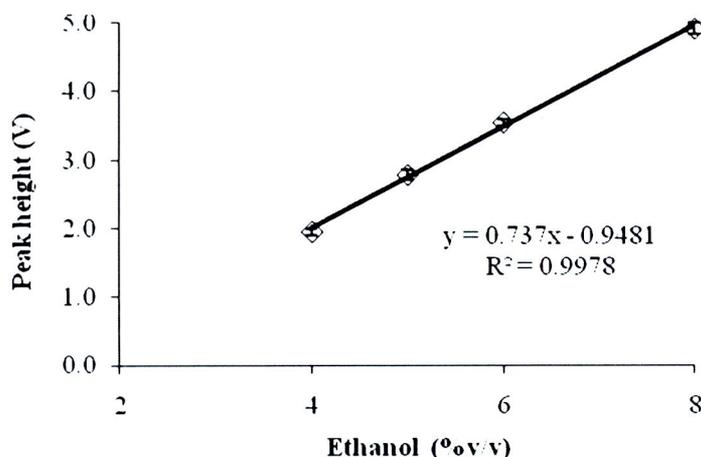


Figure 24 Calibration graph for the determination of ethanol (n=3) by using acidic $K_2Cr_2O_7$

3. Application to sample

The optimum conditions of FI-colorimetric systems was applied to determine ethanol in Thai white distilled liquor samples of different brands by using both reagent solution of an acidic potassium permanganate and acidic potassium dichromate. The ethanol standard solution of all studies was added into all real samples at two different concentration level (15 %v/v for using acidic potassium permanganate reagent solution and 2 %v/v for using acidic potassium dichromate reagent solution). Each sample solution was analysed in triplicate. The results were compared by other methods of FI-spectrophotometric, AOAC redox titration and micro-scale potentiometric redox titration and evaluated by t-test.

For the used of an acidic potassium permanganate as reagent solution, the results are shown in Table 18 and Figure 25. The different between the means and the precisions obtained from the FI- spectrophotometric system, AOAC redox titration and a micro-scale potentiometric redox titration were evaluated by t-test. The calculated t-test value is 0.79, 1.70 and 0.41 respectively. The critical value of t-test is 3.18 (where t has 3 degrees of freedoms) at the confidence interval of 95% and since the calculated value of t-test value is less than the critical value. These results from the four recommended methods are not significantly different for the mean ethanol concentration at confidence interval of 95%. Recoveries calculation from added concentration was in range of 102-104%.

For the used of an acidic potassium dichromate as reagent solution, the results are shown in Table 19 and Figure 26. The different between the means and the precisions obtained from the FI- spectrophotometric system, AOAC redox titration and a micro-scale potentiometric redox titration were also evaluated by t-test. The calculated t-test value is 1.02, 0.20 and 1.08 in which the critical value of t-test is 2.78, 2.20 and 2.20 (where t has 4, 11 and 11 degrees of freedoms), respectively, at the confidence interval of 95%. And since the calculated value of t-test value is less than the critical value, these results from the four recommended methods are not significantly different for the mean ethanol concentration at confidence interval of 95%. Recoveries calculation from added concentration was in the range of 98-105%.

Table 18 Determination of ethanol in Thai white distilled liquor by using acidic KMnO_4 reagent solution

Sample No. *	Label (%v/v)	Added (%v/v)	Ethanol found (%v/v); n=3				
			FI-colorimetric	FI-spectrophotometric	AOAC redox titration	Micro-scale potentiometric redox titration	
			$\bar{X} \pm \text{SD}^{**}$	% Recovery ^{***}	$\bar{X} \pm \text{SD}$	$\bar{X} \pm \text{SD}$	$\bar{X} \pm \text{SD}$
1	30	-	29.9±0.3	-	29.1±0.0	30.4±0.1	30.6±0.0
2	40	-	41.2±0.5	-	39.7±0.0	40.4±0.2	39.6±0.0
3	40	0	32.8±0.2	-	31.2±1.2	32.0±0.2	33.2±0.1
		15	48.6±1.0	102±10	-	-	-
4	40	0	39.1±0.2	-	36.4±0.0	38.4±0.1	40.2±0.1
		15	54.8±0.8	104±8	-	-	-

* Sample 1 and 2 synthetic samples of standard ethanol solution in water

** Average value ± standard deviation of triplicate results.

*** %Recovery = (concentration found / concentration added) × 100

Table 19 Determination of ethanol in Thai white distilled liquor by using acidic $K_2Cr_2O_7$ reagent solution

Sample No. *	Label (%v/v)	Added (%v/v)	Ethanol found (%v/v); n=3				
			FI-colorimetric		FI-spectrophotometric	AOAC redox titration	Micro-scale potentiometric redox titration
			$\bar{X} \pm SD^{**}$	% Recovery ^{***}	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
1	30	-	30.1±0.1	-	-	30.4±0.1	30.6±0.0
2	40	-	39.9±0.1	-	-	40.4±0.2	39.6±0.0
3	45	-	45.0±0.1	-	-	45.2±0.2	44.3±0.0
4	40	0	38.3±0.1	-	-	36.6±0.2	36.8±0.2
		2	40.3±0.1	103±6	-	-	-
5	40	0	39.6±0.1	-	-	40.0±0.2	39.5±0.2
		2	41.5±0.1	98±7	-	-	-
6	40	0	39.0±0.2	-	-	39.7±0.2	40.2±0.0
		2	41.0±0.1	101±9	-	-	-
7	40	0	38.8±0.0	-	-	40.3±0.1	40.5±0.2
		2	40.8±0.1	100±9	-	-	-
8	40	0	39.1±0.1	-	37.3±0.3	39.3±0.2	39.4±0.0
		2	41.1±0.1	102±6	-	-	-
9	40	0	33.5±0.1	-	32.5±0.2	32.0±0.2	33.2±0.1
		2	35.5±0.1	102±7	-	-	-
10	40	0	40.2±0.1	-	40.2±0.2	38.4±0.1	40.2±0.1
		2	42.3±0.1	102±8	-	-	-
11	40	0	39.6±0.3	-	40.9±0.4	40.9±0.1	41.3±0.0
		2	41.6±0.1	102±16	-	-	-
12	35	0	35.5±0.1	-	35.2±0.1	36.0±0.3	36.9±0.0
		2	37.6±0.0	105±3	-	-	-

* Sample 1, 2 and 3 were synthetic samples of standard ethanol solution in water

** Average value ± standard deviation of triplicate results.

*** %Recovery = (concentration found / concentration added) × 100

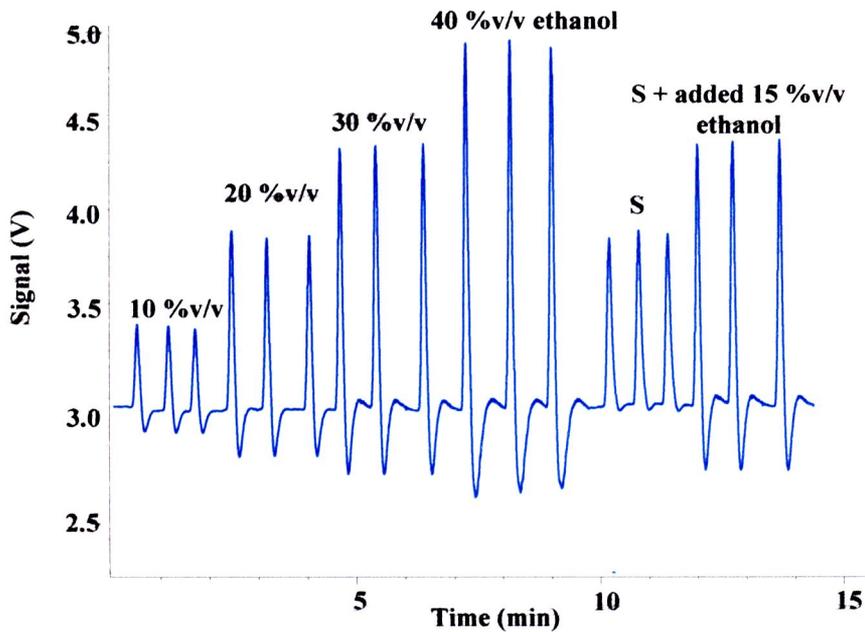


Figure 25 Typical FI-signals for ethanol determination in Thai white distilled liquor sample (S) by using FI-colorimetric system and acidic KMnO_4 reagent solution

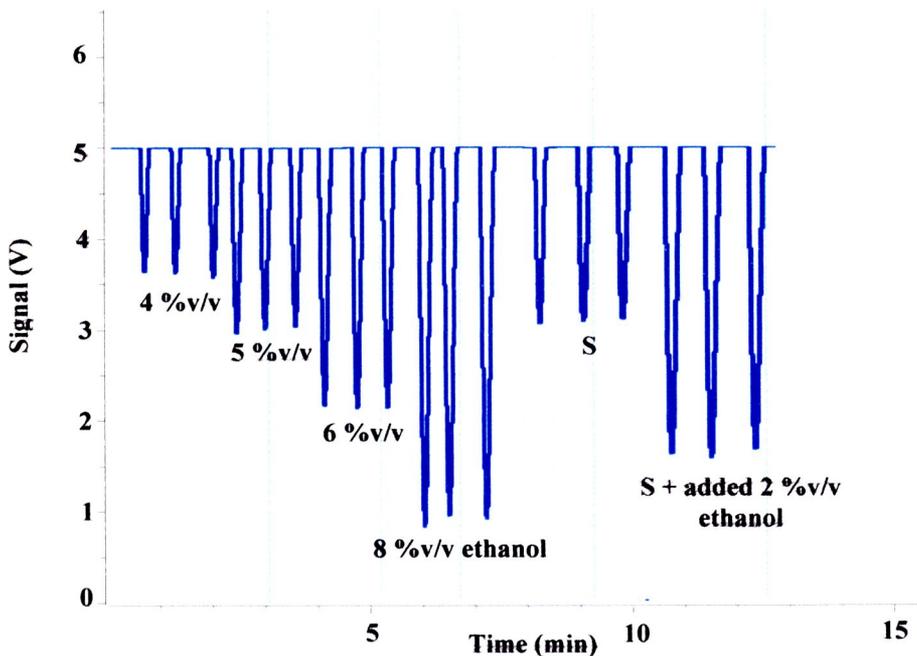


Figure 26 Typical FI signals for ethanol determination in Thai white distilled liquor sample (S) by using FI-colorimetric system and acidic $\text{K}_2\text{Cr}_2\text{O}_7$ reagent solution

Determination of ethanol in alcoholic beverage by HSI-spectrophotometric system

1. Optimization of HSI-spectrophotometric system

Preliminary conditions for ethanol determination were used as show in Table 20. And optimum conditions study for the determination of ethanol by using an acidic potassium dichromate was as followed:

Table 20 Preliminary conditions used of HSI-spectrophotometric system

Parameters	Conditions Used
HSI-spectrophotometric system:	
GD unit	
Membrane	PTFE
Membrane thickness (mm)	0.15
Carrier 1, 2 and 3	water
Reagent solutions	
R ₁	0.15 mol/L K ₂ Cr ₂ O ₇ + 4.0 mol/L H ₂ SO ₄
R ₂	0.15 mol/L K ₂ Cr ₂ O ₇ + 4.0 mol/L H ₂ SO ₄
Sample/Standard volumes	
L _{S1} (μL)	500 (80 cm, 0.89 mm i.d.)
L _{S2} (μL)	25 (5.0 cm, 0.89 mm i.d.)
Reagent volumes	
L _{R1} (μL)	25 (5.0 cm, 0.89 mm i.d.)
L _{R2} (μL)	25 (5.0 cm, 0.89 mm i.d.)
Flow rates	
Donor stream (mL/min)	0.52
Acceptor stream (mL/min)	1.0
Carrier ₁ (mL/min)	1.0
Reaction coil length (cm)	50 (0.89 mm i.d.)

**Table 20 (Cont.)**

Parameters	Conditions Used
Operation times of the system:	
Loading times	
Sample/standard at L_{S1} (s)	5
Diffuse and stopped times at GD	
Diffused at donor stream (s)	73
Stopped at acceptor stream (s)	73
Filling times	
R_1 at L_{R1} (s) to WC and W_4	10
Sample/Standard at L_{S2} (s) to WC and W_4	14
R_2 at L_{R2} (s) to WC and W_4	10
Injected/dispensed and stopped times of a sequential zone ($R_1 + \text{Sample/Standard} + R_2$)	
Injected to RC (s)	15
Stopped at RC (s)	60
Injected from RC to FC and W_4 (s)	175
Cleaning times	
Donor stream (s)	30
Acceptor stream (s)	60

1.1 Study of diffusion times, sample volumes at L_{S1} and membrane thicknesses

The diffusion time is an important parameter for ethanol diffusion from donor solution (water) into acceptor solution (water). This time depended on sample volumes at L_{S1} and flow rates of donor stream. Thus, diffusion time of the system was studied. Potassium permanganate solution (0.1 mol/L) was used for the proposed study. Using the manifold as shown in Figure 9 and preliminary conditions as shown in Table 21, various flow rates were 0.52, 0.70 and 1.15 mL/min. It was found that a flow rate of 0.70 mL/min was selected for all study of each sample volume (200, 300, 400 and 500 μL) in which diffusion times of 42, 55, 62 and 73 s were selected. After that each selected diffusion times was studied by injecting a 0.1 mol/L KMnO_4 into donor stream. The results are shown in Table 22 and Figure 27. It

was found that the increase diffusion time increased slope and the slope reached a plateau at 62 and 73 s. Using sample volume of 400 and 500 μL , respectively. Thus, a 73 s of diffusion time was chosen within 500 μL sample volume and 0.70 mL/min of flow rate of carrier at P_1 .

The membrane thickness was also studied. Using the condition of diffusion time as described above, blank and standard ethanol solutions (2 and 10 %v/v) were loaded at L_{S1} and started the operation cycle. Various thicknesses of membrane (0.075, 0.10 and 0.150 mm) were varied and optimized. The results are shown in Table 23 and Figure 28. A membrane thickness of 0.150 nm was selected as giving good precision and no easy to tear during preparation and cleaning steps

Table 21 Diffusion time with different sample volumes and flow rates of donor stream

Sample volume (μL)	Flow rate (mL/min)	Time (s)
200	0.52	55
	0.70	42
	1.15	26
300	0.52	63
	0.70	55
	1.15	32
400	0.52	85
	0.70	62
	1.15	36
500	0.52	105
	0.70	73
	1.15	43

Table 22 Effect of diffusion times and sample volumes at L_{S1} on peak height and slope of standard ethanol solutions

Diffu sion time (s)	Sam ple vo- lume (μ L)	Flow rate of donor stream (mL/min)	Etha nol (%v/v)	Peak height (V)						Slope	R^2	
				1	2	3	\bar{X}	\bar{X} - blk	SD			
42	200	0.70	0	0	0.08	0.08	0.08	0.08	0.08	0.00	0.0055	1.0000
			20	20	0.22	0.23	0.23	0.23	0.23	0.01		
			40	40	0.49	0.48	0.47	0.48	0.48	0.01		
55	300	0.70	0	0.09	0.09	0.09	0.09	0.00	0.00	0.0095	1.0000	
			20	0.27	0.28	0.29	0.28	0.19	0.01			
			40	0.65	0.66	0.67	0.66	0.38	0.01			
62	400	0.70	0	0.10	0.10	0.09	0.10	0.00	0.00	0.0145	1.0000	
			20	0.29	0.29	0.29	0.29	0.20	0.00			
			40	0.58	0.58	0.58	0.58	0.49	0.00			
73	500	0.70	0	0.08	0.07	0.08	0.08	0.00	0.01	0.0145	1.0000	
			20	0.29	0.30	0.30	0.30	0.22	0.01			
			40	0.58	0.60	0.58	0.59	0.51	0.01			

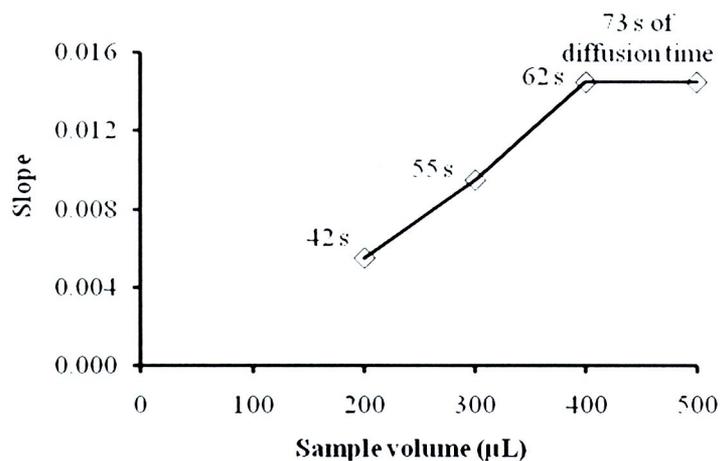


Figure 27 Effect of diffusion times and sample volumes at L_{S1} on slope of standard ethanol solutions (20 and 40 %v/v)

Table 23 Effect of membrane thicknesses on peak height of standard ethanol solutions

Thickness of membrane (mm)	Ethanol (%v/v)	Peak height (V)					
		1	2	3	\bar{X}	\bar{X} -blk	SD
0.075	0	1.57	1.54	1.54	1.55	0.00	0.02
	2	1.77	1.65	2.01	1.81	0.26	0.18
0.100	0	1.61	1.55	1.56	1.57	0.00	0.03
	2	2.31	2.38	2.39	2.36	0.79	0.05
	10	5.31	5.34	5.30	5.32	3.74	0.02
0.150	0	1.51	1.51	1.51	1.51	0.00	0.00
	2	1.95	1.94	1.95	1.95	0.44	0.01
	10	4.36	4.36	4.39	4.37	2.86	0.02

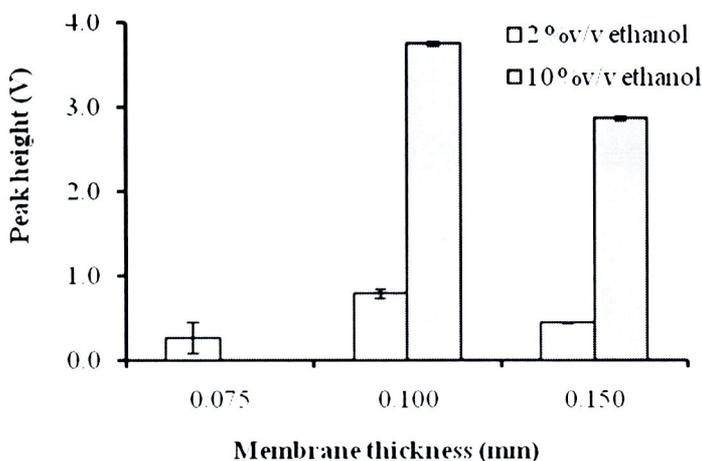


Figure 28 Effect of membrane thicknesses on slope of standard ethanol solutions (2 and 10 %v/v)

1.2 Effect of filling times of diffuse zone containing ethanol

Filling time of diffuse zone containing ethanol from acceptor stream into L_{S2} is effect to sensitivity of ethanol determination. Thus, the effect of filling time of diffuse zone containing ethanol was studied. Using conditions described in 1.1, blank and standard ethanol solution (2 %v/v) were loaded at L_{S1} and started the operation cycle. Various aspirated times (10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30

s) were varied and optimized. The results are shown in Table 24 and Figure 29. It was found that the filling time at 14 s was chosen as giving the highest peak height which indicated the highest ethanol concentration in this zone.

Table 24 Effect of filling times of diffuse zone containing ethanol on peak height of 2 %v/v ethanol

Filling time of diffuse zone (s)	Peak height (V)			
	1	2	\bar{X}	SD
10	0.39	0.39	0.39	0.00
12	0.46	0.46	0.46	0.00
14	0.55	0.54	0.55	0.01
16	0.51	0.51	0.51	0.00
18	0.51	0.51	0.51	0.00
20	0.48	0.49	0.49	0.00
22	0.47	0.49	0.49	0.01
24	0.45	0.47	0.46	0.02
26	0.39	0.39	0.39	0.00
28	0.37	0.38	0.38	0.01
30	0.32	0.33	0.32	0.01

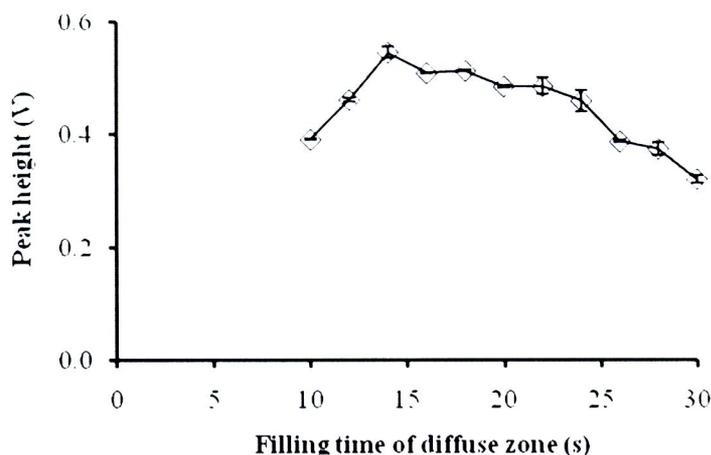


Figure 29 Effect of filling times of diffuse zone containing ethanol on slope of 2 %v/v ethanol

1.3 Effect of H₂SO₄ concentrations

Sulfuric acid is a portion of the reaction and its effect to sensitivity and linear range of calibration graph for ethanol determination. Thus, the effect of H₂SO₄ concentrations was studied. Using conditions described in 1.2, blank and standard ethanol solutions (2, 4, 6, 8, 10, 20, 30 and 40 %v/v) were loaded at L_{S1} and started the operation cycle. Various concentration of H₂SO₄ (1.0, 2.0, 3.0 and 4.0 mol/L) were varied and optimized. The results are shown in Table 25 and Figure 30. The increase H₂SO₄ concentration increased peak height. Linear ranges of calibration graphs were found 20-40 %v/v ethanol, 10-40 %v/v ethanol, 2-10 and 10-40 %v/v ethanol and 2-10 and 10-40 %v/v ethanol for 1.0, 2.0, 3.0 and 4.0 mol/L H₂SO₄, respectively. Moreover, high H₂SO₄ concentration produced reagent bubbles. To compromise between sensitivity, less reagent bubbles and expense of chemicals, the H₂SO₄ concentration of 4.0 mol/L was selected for further studies.

Table 25 Effect of H₂SO₄ concentrations on peak height and slope of standard ethanol solutions

H ₂ SO ₄ (mol/L)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
1.0	0	0.06	0.06	0.06	0.06	0.00	0.00	0.0012	0.9624
	2	0.06	0.06	0.06	0.06	0.00	0.00		
	4	0.06	0.06	0.06	0.06	0.00	0.00		
	6	0.06	0.06	0.06	0.06	0.00	0.00		
	8	0.06	0.06	0.06	0.06	0.00	0.00		
	10	0.08	0.08	0.08	0.08	0.02	0.00		
	20	0.08	0.08	0.08	0.08	0.02	0.00		
	30	0.09	0.10	0.09	0.09	0.03	0.00		
	40	0.10	0.10	0.10	0.10	0.04	0.00		
2.0	0	0.08	0.08	0.08	0.08	0.00	0.00		
	2	0.08	0.08	0.08	0.08	0.00	0.00		
	4	0.09	0.09	0.09	0.09	0.02	0.00		
	6	0.10	0.10	0.10	0.10	0.02	0.00		

Table 25 (Cont.)

H ₂ SO ₄ (mol/L)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
2.0	8	0.15	0.14	0.15	0.15	0.06	0.00	0.0029	0.9970
	10	0.18	0.18	0.18	0.18	0.09	0.00		
	20	0.21	0.21	0.21	0.21	0.12	0.00		
	30	0.26	0.25	0.26	0.26	0.17	0.00		
	40	0.31	0.31	0.31	0.31	0.22	0.00		
3.0	0	0.09	0.09	0.09	0.09	0.00	0.00	0.0100	0.9767
	2	0.10	0.10	0.10	0.10	0.01	0.00		
	4	0.11	0.11	0.11	0.11	0.02	0.00		
	6	0.13	0.13	0.13	0.13	0.04	0.00		
	8	0.15	0.14	0.15	0.15	0.06	0.00		
	10	0.18	0.18	0.18	0.18	0.09	0.00		
	20	0.21	0.21	0.21	0.21	0.12	0.00		
	30	0.26	0.25	0.26	0.26	0.17	0.00		
	40	0.31	0.31	0.31	0.31	0.22	0.00		
4.0	0	0.10	0.10	0.10	0.10	0.00	0.00	0.0102	0.9582
	2	0.12	0.12	0.12	0.12	0.02	0.00		
	4	0.13	0.13	0.13	0.13	0.03	0.00		
	6	0.15	0.15	0.15	0.15	0.05	0.00		
	8	0.20	0.19	0.19	0.19	0.09	0.01		
	10	0.20	0.21	0.20	0.20	0.10	0.01		
	20	0.33	0.33	0.34	0.33	0.23	0.00		
	30	0.43	0.45	0.43	0.43	0.33	0.01		
	40	0.53	0.56	0.55	0.55	0.44	0.02		

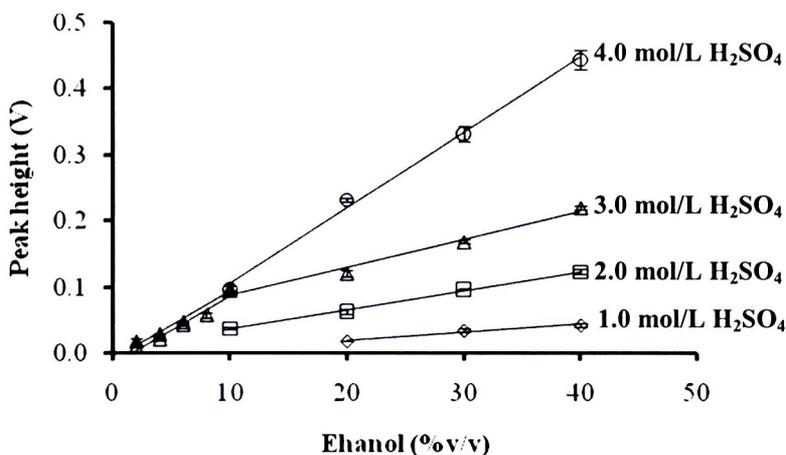


Figure 30 Effect of H₂SO₄ concentrations on peak height of standard ethanol solutions (2, 4, 6, 8, 10, 20, 30 and 40 %v/v)

1.4 Effect of K₂Cr₂O₇ concentrations

Potassium dichromate is an oxidize ethanol and it is effect to sensitivity and linear range of calibration graph for ethanol determination. Thus, the effect of K₂Cr₂O₇ concentration was studied. Using conditions described in 1.3, blank and standard ethanol solutions (2, 4, 6, 8, 10, 20, 30 and 40 %v/v) were loaded at L_{S1} and started the operation cycle. Various concentration of K₂Cr₂O₇ (0.05, 0.10, 0.15 and 0.2 mol/L) were varied and optimized. The results are shown in Table 26 and Figure 31. The increase K₂Cr₂O₇ concentration increased peak height. Linear ranges of calibration graphs were found 4-40 %v/v ethanol for 0.05 mol/L K₂Cr₂O₇ and 2-10 %v/v and 10-40 %v/v ethanol for 0.10, 0.15 and 0.20 mol/L of K₂Cr₂O₇, respectively. Therefore, K₂Cr₂O₇ concentration at 0.20 mol/L was chosen as giving the highest peak height and slope (in a linear range of 2-10 %v/v ethanol) though this concentration was spent long time to dissolve in water. And calibration graph in the range of 2-10 %v/v ethanol was selected for all further studies.

Table 26 Effect of $K_2Cr_2O_7$ concentrations on peak height and slope of standard ethanol solutions

$K_2Cr_2O_7$ (mol/L)	Ethanol (%v/v)	Peak height (V)						Slope	R^2
		1	2	3	\bar{X}	\bar{X} -blk	SD		
0.05	0	0.08	0.08	0.08	0.08	0.00	0.00	0.0053	0.9982
	2	0.08	0.08	0.08	0.08	0.00	0.00		
	4	0.09	0.09	0.09	0.09	0.01	0.00		
	6	0.11	0.10	0.10	0.10	0.03	0.00		
	8	0.11	0.12	0.12	0.11	0.04	0.01		
	10	0.12	0.13	0.12	0.12	0.05	0.01		
	20	0.19	0.18	0.17	0.18	0.10	0.01		
	30	0.21	0.23	0.23	0.23	0.15	0.01		
	40	0.28	0.29	0.28	0.29	0.21	0.00		
0.10	0	0.08	0.08	0.08	0.08	0.00	0.00	0.0088 0.0060	0.9631 0.9982
	2	0.10	0.10	0.10	0.10	0.02	0.00		
	4	-	-	-	-	-	-		
	6	0.12	0.12	0.12	0.12	0.04	0.00		
	8	0.15	0.15	0.15	0.15	0.07	0.00		
	10	0.17	0.17	0.16	0.17	0.09	0.00		
	20	0.23	0.23	0.24	0.23	0.15	0.00		
	30	0.30	0.29	0.28	0.29	0.21	0.01		
	40	0.33	0.35	0.36	0.35	0.27	0.01		
0.15	0	0.10	0.10	0.10	0.10	0.00	0.00	0.0156 0.0140	0.9876 0.9994
	2	0.11	0.10	0.11	0.10	0.01	0.00		
	4	0.13	0.12	0.13	0.13	0.03	0.00		
	6	0.17	0.17	0.18	0.17	0.08	0.00		
	8	0.20	0.20	0.20	0.20	0.10	0.00		
	10	0.22	0.23	0.23	0.23	0.13	0.00		
	20	0.35	0.35	0.37	0.35	0.26	0.01		
	30	0.50	0.49	0.51	0.50	0.40	0.01		
	40	0.64	0.62	0.68	0.64	0.55	0.03		

Table 26 (Cont.)

K ₂ Cr ₂ O ₇ (mol/L)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
0.20	0	0.12	0.12	0.12	0.12	0	0.00	0.0209	0.9980
	2	0.15	0.15	0.15	0.15	0.03	0.00		
	4	-	-	-	-	-	-		
	6	0.23	0.23	0.23	0.23	0.11	0.00		
	8	0.27	0.27	0.27	0.27	0.15	0.00		
	10	0.32	0.31	0.32	0.32	0.20	0.00		
	20	0.51	0.49	0.48	0.49	0.37	0.02		
	30	0.62	0.61	0.65	0.63	0.51	0.02		
	40	0.75	0.75	0.72	0.74	0.62	0.02		

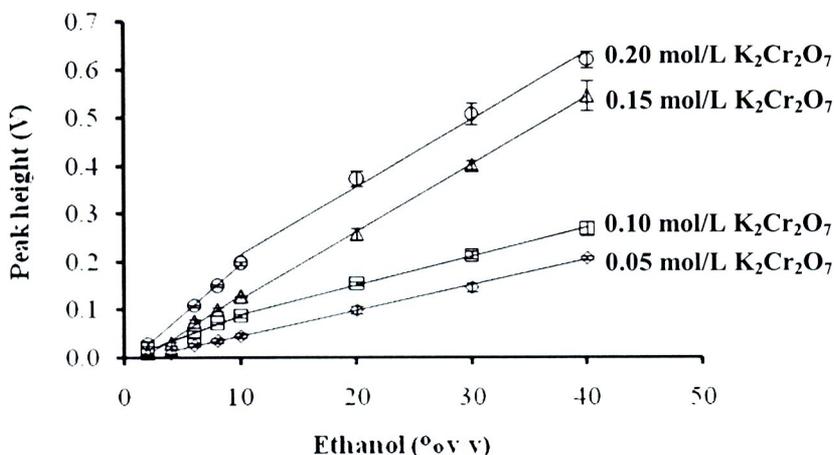


Figure 31 Effect of K₂Cr₂O₇ concentrations on peak height of standard ethanol solutions (2, 4, 6, 8, 10, 20, 30 and 40 %v/v)

1.5 Effect of reaction coil lengths

In order to achieve good mixing of sample and reagent solution and high sensitivity, the effect of reaction coil length (teflon tubing, 0.89 mm i.d.) was studied. Using conditions described in 1.4, blank and standard ethanol solutions (2, 4, 6, 8 and 10 %v/v) were loaded at L_{S1} and started the operation cycle. Various reaction coil length (20, 30, 40 and 50 cm) were varied and optimized. The results are shown in

Table 27 and Figure 32. It was found that a reaction coil of 30 cm was chosen as giving the highest peak height and slope which indicated a good mixing of sample and reagent solutions.

Table 27 Effect of reaction coil lengths on peak height and slope of standard ethanol solutions

Reaction coil length (cm)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
20	0	0.25	0.26	0.25	0.25	0.00	0.00	0.0166	0.0634
	2	0.27	0.27	0.27	0.27	0.02	0.00		
	4	0.29	0.29	0.29	0.29	0.04	0.00		
	6	0.31	0.32	0.32	0.31	0.06	0.01		
	8	0.37	0.36	0.36	0.36	0.11	0.01		
	10	0.40	0.40	0.41	0.40	0.15	0.00		
30	0	0.16	0.16	0.16	0.16	0.00	0.00	0.0287	0.9935
	2	0.18	0.18	0.18	0.18	0.02	0.00		
	4	0.21	0.22	0.21	0.22	0.06	0.01		
	6	0.29	0.29	0.29	0.29	0.13	0.00		
	8	0.35	0.33	0.35	0.35	0.19	0.01		
	10	0.39	0.41	0.39	0.40	0.24	0.01		
40	0	0.15	0.15	0.15	0.15	0.00	0.00	0.0173	0.9908
	2	0.16	0.16	0.16	0.16	0.01	0.00		
	4	0.20	0.20	0.20	0.20	0.05	0.00		
	6	0.21	0.21	0.23	0.22	0.07	0.01		
	8	0.27	0.27	0.25	0.27	0.12	0.01		
	10	0.29	0.29	0.29	0.29	0.14	0.00		
50	0	0.08	0.07	0.07	0.07	0.00	0.00	0.0104	0.9938
	2	0.12	0.12	0.12	0.12	0.04	0.00		
	4	0.13	0.13	0.14	0.13	0.06	0.01		
	6	0.16	0.16	0.16	0.16	0.08	0.00		
	8	0.18	0.18	0.18	0.18	0.10	0.00		
	10	0.19	0.20	0.20	0.20	0.12	0.01		

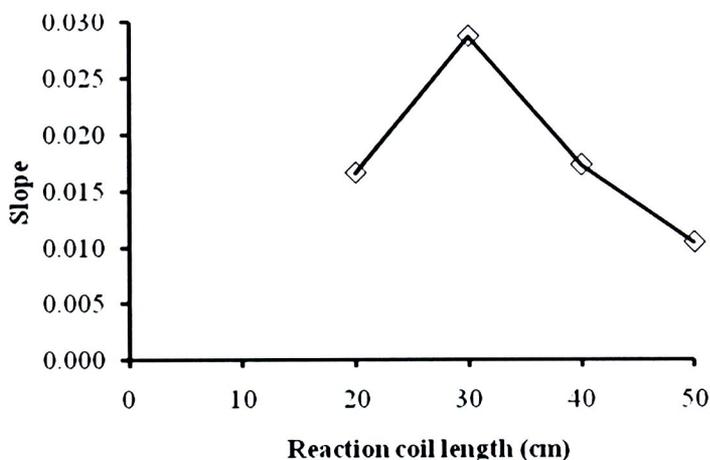


Figure 32 Effect of reaction coil lengths on slope of standard ethanol solutions (2, 4, 6, 8 and 10 %v/v)

1.6 Effect of sample/standard volumes at L_{S2}

The effect of sample/standard volume at L_{S2} (teflon tubing, 0.89 mm i.d) was studied because of effective dispersion and sensitivity of the system. Using conditions described in 1.5, blank and standard ethanol solutions (2 and 10 %v/v) were loaded at L_{S1} and started the operation cycle. Various sample/standard volume at L_{S2} (16, 25 and 38 μL) were varied and optimized. The results are shown in Table 28 and Figure 33. The results indicate that increase in sample volume increased peak heights and slope volume of 38 μL was chosen.

Table 28 Effect of sample/standard volumes at L_{S2} on peak height and slope of standard ethanol solution

Sample/ Standard volume at L_{S2} (μL)	Length of L_{S2} (cm)	Ethanol (%v/v)	Peak height (V)						Slope	R^2
			1	2	3	\bar{X}	\bar{X} -blk	SD		
		0	0.12	0.12	0.12	0.12	0.00	0.00		
16	2.5	2	0.14	0.15	0.14	0.14	0.02	0.01	0.0212	1.0000
		10	0.31	0.30	0.31	0.31	0.19	0.01		

Table 28 (Cont.)

Sample/ Standard volume at L_{S2} (μL)	Length of L_{S2} (cm)	Ethanol (%v/v)	Peak height (V)						Slope	R^2
			1	2	3	\bar{X}	\bar{X} -blk	SD		
16	2.5	0	0.12	0.12	0.12	0.12	0.00	0.00	0.0212	1.0000
		2	0.14	0.15	0.14	0.14	0.02	0.01		
		10	0.31	0.30	0.31	0.31	0.19	0.01		
25	5.0	0	0.12	0.12	0.12	0.12	0.00	0.00	0.0215	1.0000
		2	0.16	0.16	0.17	0.16	0.04	0.01		
		10	0.33	0.33	0.33	0.33	0.21	0.00		
38	7.5	0	0.14	0.13	0.13	0.13	0.00	0.01	0.0229	1.0000
		2	0.15	0.15	0.14	0.15	0.02	0.00		
		10	0.33	0.33	0.34	0.33	0.20	0.01		

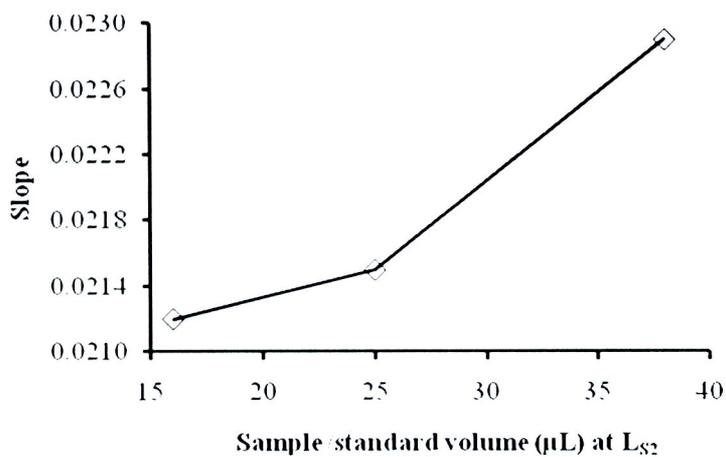


Figure 33 Effect of slope/standard volumes at L_{S2} on slope of standard ethanol solutions (2 and 10 %v/v)

1.7 Effect of stopped times at reaction coil

The effect of stopped times at reaction coil (RC) was studied to optimize a completeness of reaction and sensitivity. Using conditions described in 1.6, blank and standard ethanol solutions (2, 4, 6 and 10 %v/v) were loaded at L_{S1} and started the operation cycle. Various stopped times (40, 60, 80 and 100 min) were varied and optimized. The results are shown in Table 29 and Figure 34. The results indicate that slope reached a plateau at 60-100 min of stopped time. Below 60 min, the decrease in peak height and slope was small due to no complete reaction. A stopped time of 60 s was selected for further studies.

Table 29 Effect of stopped times at reaction coil on peak height and slope of standard ethanol solutions

Stopped time (s)	Ethanol (%v/v)	Peak height (V)						Slope	R ²
		1	2	3	\bar{X}	\bar{X} -blk	SD		
40	0	0.38	0.39	0.37	0.38	0.00	0.01	0.0538	0.9864
	2	0.40	0.43	0.42	0.41	0.04	0.02		
	4	0.50	0.49	0.50	0.49	0.11	0.00		
	6	0.65	0.66	0.66	0.65	0.27	0.01		
	10	0.84	0.82	0.84	0.83	0.45	0.01		
60	0	0.34	0.33	0.34	0.34	0.00	0.01	0.0596	0.9999
	2	0.40	0.41	0.41	0.41	0.07	0.00		
	4	0.53	0.53	0.51	0.52	0.18	0.01		
	6	0.64	0.64	0.65	0.65	0.31	0.00		
	10	0.90	0.89	0.85	0.88	0.54	0.03		
80	0	0.28	0.29	0.29	0.29	0.00	0.01	0.0582	0.0071
	2	0.45	0.44	0.43	0.44	0.15	0.01		
	4	0.58	0.60	0.56	0.58	0.29	0.02		
	6	0.68	0.68	0.70	0.69	0.40	0.01		
	10	0.90	0.93	0.90	0.91	0.62	0.02		
100	0	0.28	0.29	0.29	0.29	0.00	0.01	0.0597	0.9993
	2	0.45	0.44	0.45	0.45	0.16	0.00		
	4	0.58	0.59	0.56	0.58	0.29	0.02		
	6	0.68	0.68	0.70	0.69	0.40	0.01		
	10	0.90	0.93	0.95	0.93	0.64	0.03		

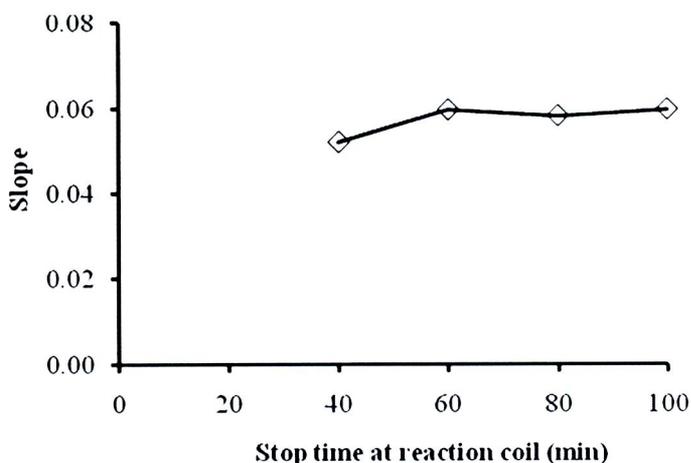


Figure 34 Effect of stopped times at reaction coil on slope of standard ethanol solutions (2, 4, 6, 8 and 10 %v/v)

1.8 Summary of conditions used

The recommended HSI-spectrophotometric manifold is depicted in Figure 9 and the optimum conditions are summarized in Table 30.

Table 30 Conditions used for the determination of ethanol

Parameters	Conditions Used
HSI-spectrophotometric system:	
GD unit	
Membrane	PTFE
Membrane thickness (mm)	0.15
Carrier 1,2 and 3	water
Reagent solutions	
R ₁	0.2 mol/L K ₂ Cr ₂ O ₇ + 4.0 mol/L H ₂ SO ₄
R ₂	0.2 mol/L K ₂ Cr ₂ O ₇ + 4.0 mol/L H ₂ SO ₄
Sample/Standard volumes	
L _{S1} (μL)	500 (80 cm, 0.89 mm i.d.)
L _{S2} (μL)	38 (7.5 cm, 0.89 mm i.d.)

Table 30 (Cont.)

Parameters	Conditions Used
Reagent volumes	
L _{R1} (μL)	25 (5.0 cm, 0.89 mm i.d.)
L _{R2} (μL)	25 (5.0 cm, 0.89 mm i.d.)
Flow rates	
Donor stream (mL/min)	0.70
Acceptor stream (mL/min)	1.0
Carrier ₁ (mL/min)	1.0
Reaction coil length (cm)	30 (0.89 mm i.d.)
Operation times of the system:	
Loading times	
Sample/standard at L _{S1} (s)	5
Diffuse and stopped times at GD	
diffused at donor stream (s)	73
Stopped at acceptor stream (s)	73
Filling times	
R ₁ at L _{R1} (s) to WC and W ₄	10
Sample/Standard at L _{S2} (s) to WC and W ₄	16
R ₂ at L _{R2} (s) to WC and W ₄	10
Injected/dispensed and stopped times of a sequential zone (R₁ + Sample/Standard + R₂)	
Injected to RC (s)	15
Stopped at RC (s)	60
Injected from RC to FC and W ₄ (s)	175
Cleaning times	
Donor stream (s)	30
Acceptor stream (s)	60

1.9 Interference study

The effect of interference compounds (including other alcohols, sugars, ethyl acetate and acetaldehyde that could be maximum found in samples) was studied. Using conditions described in 1.1-1.8, blank and standard ethanol solution (6 %v/v or 47400 mg/L) containing various interferences with various concentrations were loaded at L_{S1} and started the operation cycle. The results are shown in Table 31. It

was found that interference compounds which could be maximum found in samples were did not interfered (defined as a relative error of $< \pm 5\%$ in the signal of 6 %v/v ethanol) for ethanol determination. That means these interference compounds were not seriously interfered in this method and not significantly affected for ethanol determination in alcoholic beverage samples (beer, wine, brandy and Thai white distilled liquor) because these samples contained very low concentrations of these interferences.

Table 31 Effect of interference on peak height of 6 %v/v (or 47400 mg/L); mean of triplicate injections

Interference compounds	Concentration added (mg/L)	Peak height (V) [*]	%Relative error ^{**}
none	-	2.66	-
Acetaldehyde	50	2.66	0.00
	100	2.69	1.13
	500	2.69	1.13
2-Methylpropan-1-ol	50	2.66	0.00
	100	2.70	1.50
	500	2.74	3.01
Propan-1-ol	50	2.68	0.75
	100	2.68	0.75
	500	2.73	2.63
Ethyl acetate	50	2.69	1.13
	100	2.54	-4.51
	500	2.53	-4.89
Ethanoic acid	500	2.74	3.01
	1000	2.74	3.01
	2000	2.65	-0.38
Glucose	500	2.62	-1.50
	1000	2.62	-1.50
	1500	2.58	-3.01



Table 31 (Cont.)

Interference compounds	Concentration added (mg/L)	Peak height (V)*	%Relative error**
Fructose	500	2.71	1.88
	1000	2.70	1.50
	2500	2.68	0.75

* Peak height was corrected from blank signal (blank = 1.42 V)

** %Relative error = $\left[\frac{(\text{peak height of of 6\%v/v ethanol+interference}) - (\text{peak height of 6\%v/v ethanol alone})}{\text{peak height of 6\%v/v ethanol alone}} \right] \times 100$

1.10 Calibration graph and detection limit

Using the manifold as shown in Figure 9 and selected conditions as studied in 1.1-1.9, blank and standard ethanol solutions (2, 4, 6, 8 and 10 %v/v) were loaded at L_{S1} and started the operation cycle. A calibration graph was plotted between peak heights obtained versus standard ethanol concentrations. A detection limit was calculated using Miller & Miller method (described in Appendix B.1). The results are shown in Table 32, Figure 35 and Figure 36. Linearity was obtained in the range of 2-10 %v/v. The detection limit was 0.4 %v/v ethanol. The RSD was in the range of 0.7-2.3 % and the sample throughput was 15 injections per hour.

Table 32 Calibration data for ethanol determination

Ethanol (%v/v)	Peak height (V)						
	1	2	3	\bar{X}	\bar{X} -blk	SD	%RSD
0	1.51	1.51	1.51	1.51	0.00	0.00	-
2	1.95	1.94	1.95	1.95	0.44	0.01	2.3
4	2.52	2.55	2.52	2.53	1.02	0.02	2.0
6	3.21	3.19	3.24	3.21	1.70	0.03	1.8
10	4.36	4.36	4.39	4.37	2.86	0.02	0.7

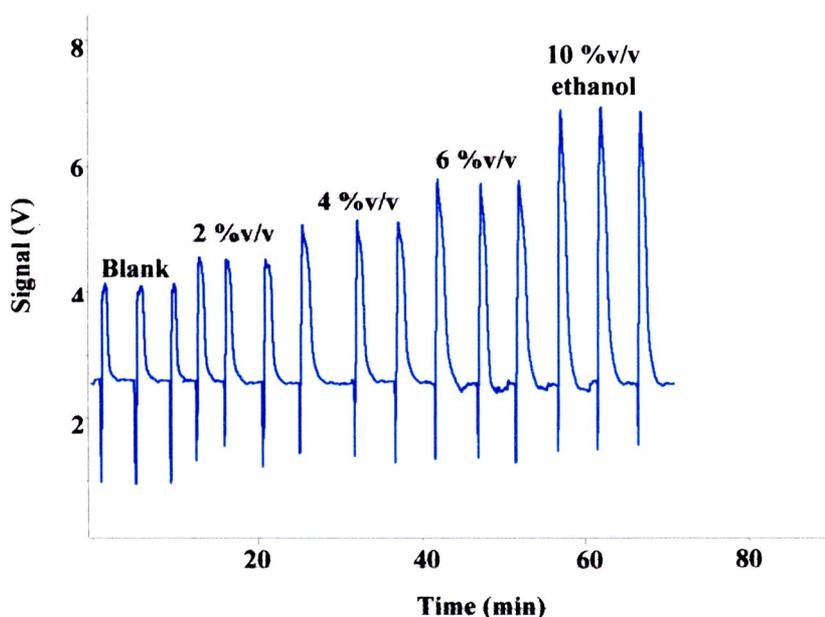


Figure 35 HSI signals for ethanol determination

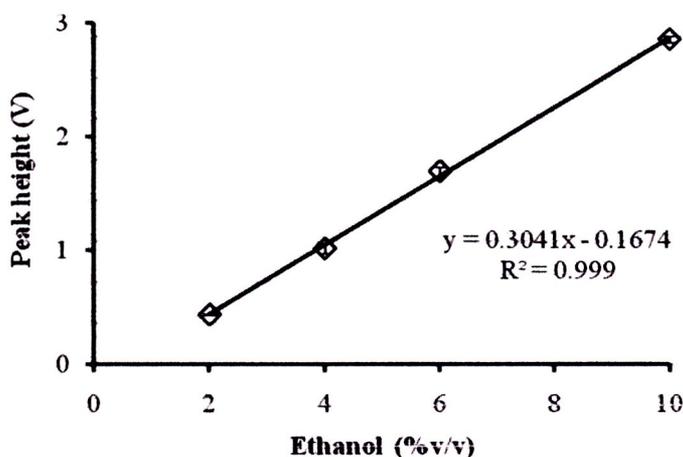


Figure 36 Calibration graph for ethanol determination (n=3) by HSI-spectrophotometric system

1.11 Application to sample

The optimum conditions of HSI-spectrophotometric system was applied to determine ethanol in different brands of alcoholic beverages including beer, wine, brandy, whisky, rum and Thai white distilled liquor samples of different brands by using acidic potassium dichromate reagent solution. The ethanol standard solution

(4 %v/v) was added into all samples. Each sample solution was analysed in triplicate. The results of samples were compared by other methods of AOAC redox titration and micro-scale potentiometric redox titration. The results are shown in Table 33 and Figure 37-41. The different between the means and the precision obtained from the HSI-spectrophotometric system, AOAC redox titration and a micro-scale potentiometric redox titration were evaluated by t-test. The calculated t-test value is 0.70 and 1.20, respectively. The critical value of t-test is 2.78 (where t has 4 degrees of freedoms) at the confidence interval of 95% and since the calculated value of t-test value is less than the critical value. These results from the four recommended methods are not significantly different for the mean ethanol concentration at confidence interval of 95%. Recoveries calculation from added concentration was in range of 97-103%.

Table 33 Determination of ethanol in alcoholic beverages

Samples No.	Alcoholic beverages	Label (%v/v)	Added (%v/v)	Ethanol found (%v/v); n=3			
				HSI-spectrophotometric	AOAC redox titration	Micro-scale potentiometric redox titration	
				$\bar{X}\pm SD^*$	% Recovery**	$\bar{X}\pm SD$	$\bar{X}\pm SD$
1	Distilled liquor 1	40	0	38.3±0.1	–	39.3±0.2	39.4±0.0
			4	42.4±0.0	101±3	–	–
2	Distilled liquor 2	40	0	34.0±0.2	–	32.0±0.2	33.2±0.1
			4	38.0±0.2	101±10	–	–
3	Distilled liquor 3	40	0	40.7±0.2	–	38.4±0.2	40.2±0.1
			4	44.7±0.4	103±9	–	–
4	Distilled liquor 4	40	0	39.3±0.2	–	40.9±0.1	41.3±0.0
			4	43.4±0.3	99±10	–	–
5	Distilled liquor 5	35	0	36.5±0.2	–	36.0±0.3	36.9±0.0
			4	40.5±0.0	99±4	–	–
6	Wine 1	10	0	9.5±0.2	–	–	–
			4	13.4±0.3	99±9	–	–
7	Wine 2	10	0	8.1±0.1	–	–	–
			4	12.0±0.2	97±6	–	–
8	Wine 3	10	0	9.8±0.1	–	–	–
			4	14.0±0.3	103±7	–	–
9	Wine4	10	0	10.2±0.2	–	–	–
			4	14.1±0.0	98±4	–	–
10	Wine5	10	0	3.4±0.0	–	–	–
			4	7.3±0.2	98±5	–	–
11	Beer 1	5	0	3.1±0.1	–	–	–
			4	7.1±0.2	99±6	–	–
12	Beer 2	6.4	0	4.1±0.1	–	–	–
			4	8.0±0.2	97±5	–	–

* Average value ± standard deviation of triplicate results.

** %Recovery = (concentration found / concentration added) × 100

Table 33 (Cont.)

Samples No.	Alcoholic beverages	Label (%v/v)	Added (%v/v)	Ethanol found (%v/v); n=3			
				HSI-spectrophotometric	AOAC redox titration	Micro-scale potentiometric redox titration	
				$\bar{X} \pm SD^*$	% Recovery**	$\bar{X} \pm SD$	$\bar{X} \pm SD$
13	Whisky	10	0	33.6±0.2	–	–	–
			4	37.8±0.2	103±6	–	–
14	Rum	40	0	38.1±0.0	–	–	–
			4	42.0±0.3	103±6	–	–
15	Brandy	38	0	37.5±0.1	–	–	–
			4	41.4±0.2	98±7	–	–

* Average value ± standard deviation of triplicate results.

** %Recovery = (concentration found / concentration added) × 100

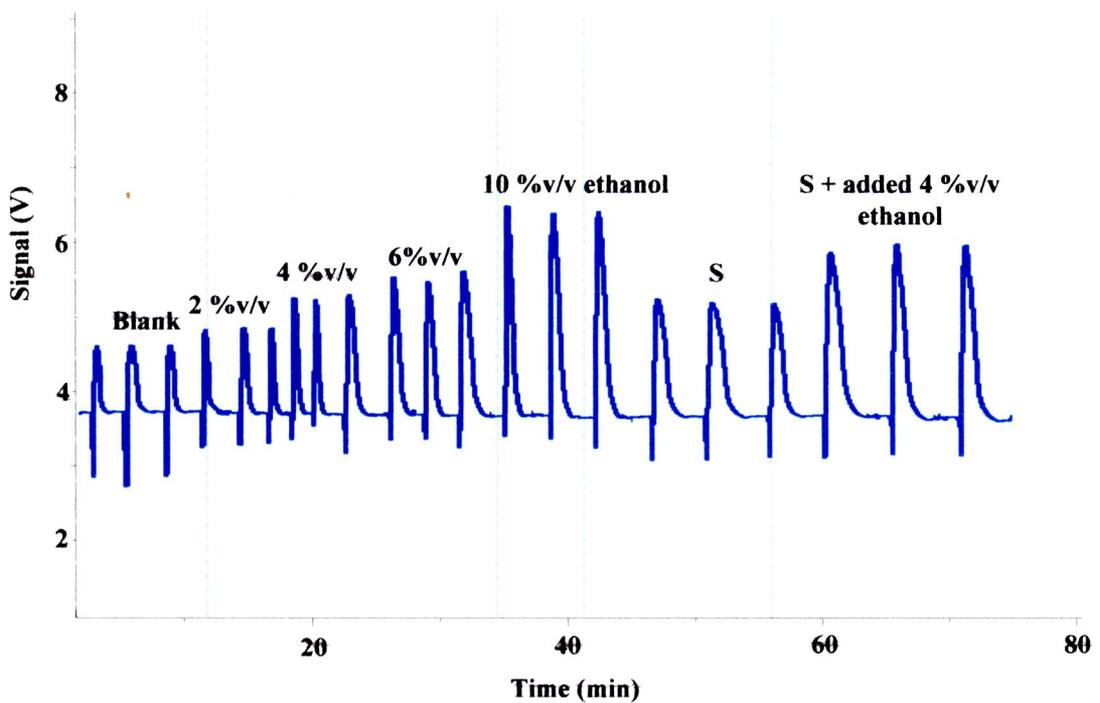


Figure 37 Typical HSI signals for ethanol determination in Thai white distilled liquor sample (S) of number 2

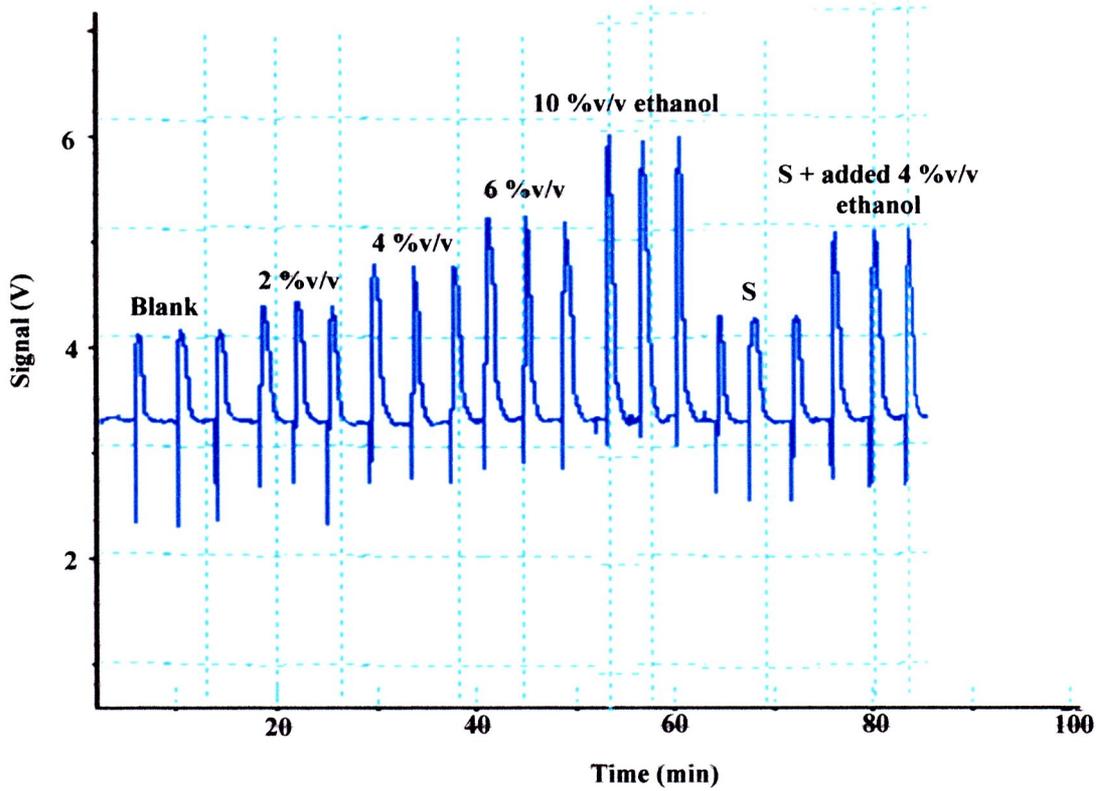


Figure 38 Typical HSI signals for ethanol determination in wine sample (S) of number 2

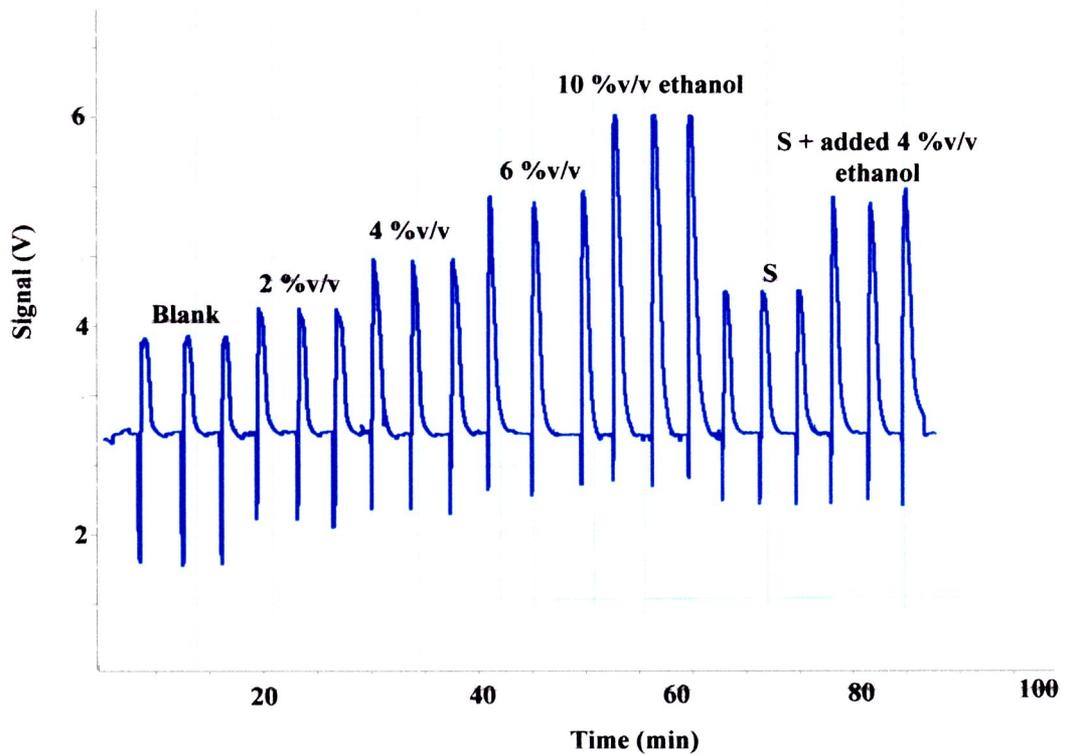


Figure 39 Typical HSI signals for ethanol determination in beer sample (S) of number 2

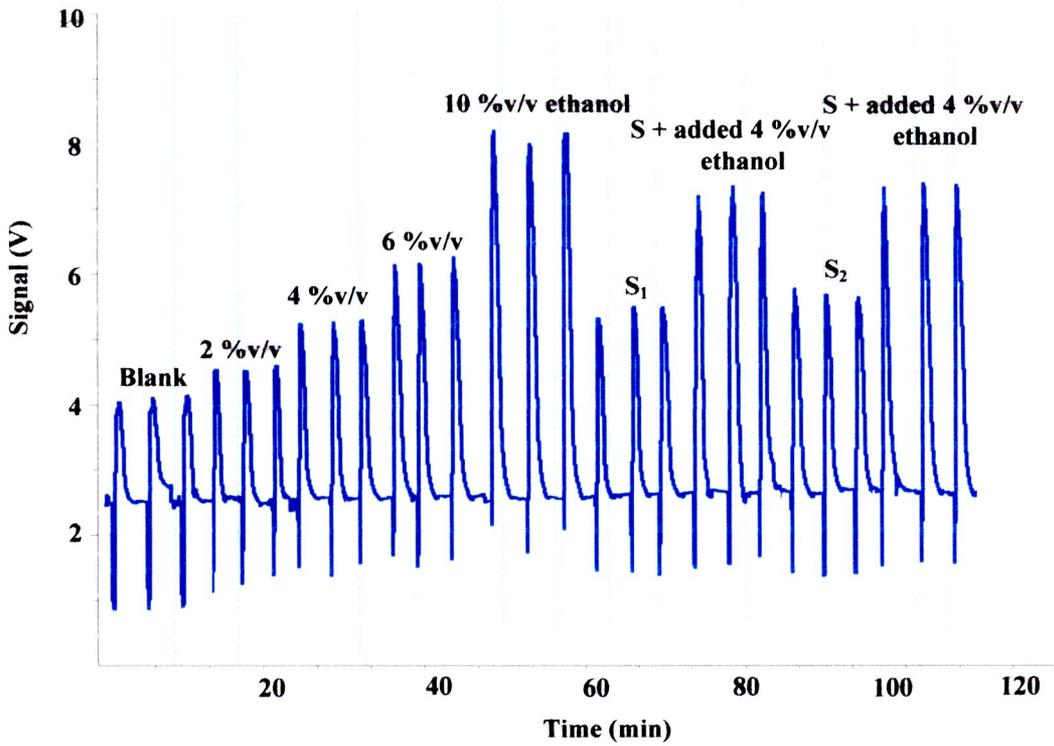


Figure 40 Typical HSI signals for ethanol determination in whisky (S₁) and brandy sample (S₂)

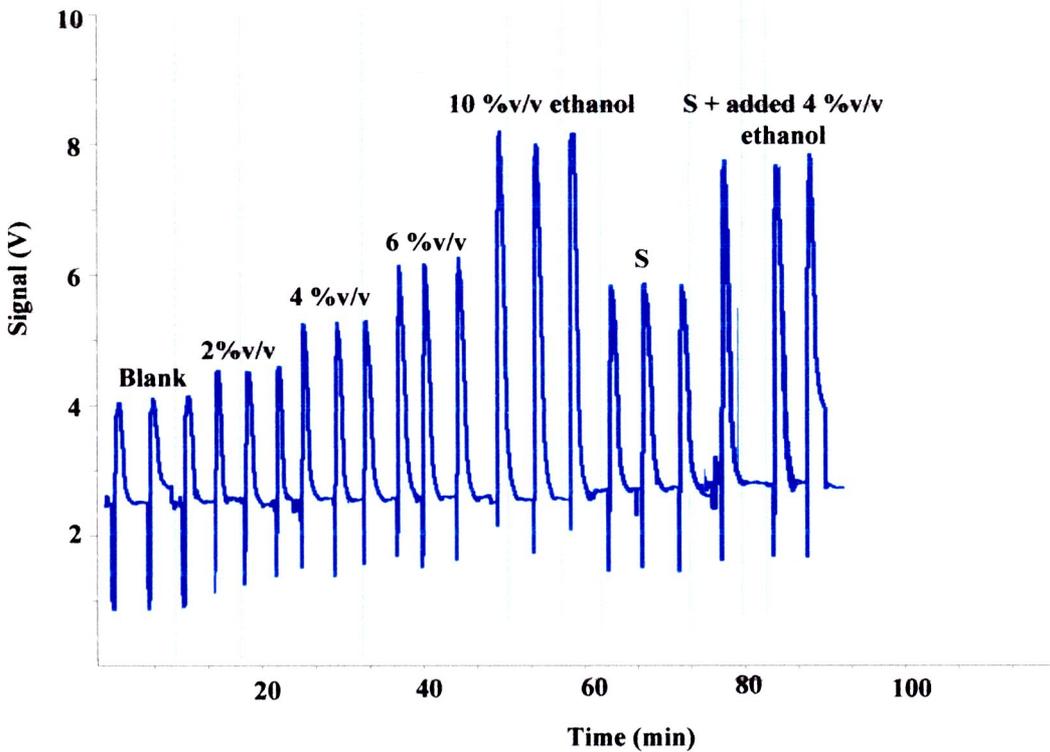


Figure 41 Typical HSI signals for ethanol determination in rum sample (S)