CHAPTER 2
EXPERIMENTAL, RESULTS AND DISCUSSION

2.1 Instruments, apparatus and chemicals

2.1.1 Instruments and apparatus

(1) Automatic SIA system analyzer (Laboratory made, Center for Biotechnology, Turku University, Finland)

The instrument suite comprised the following element:

(a) Software package (AnalySIA) for the system control

(b) Syringe pump, 2.5 mL

(c) Selection valve, seven-ports

(d) Holding coil, I.D. 0.6 mm, 200 cm

(e) Mixing coil, I.D. 0.6 mm, 100 cm

(f) Personal computer (PC), Perbi Oy, Finland

(g) Interface card, Lab-PC+, National Instrument Corperation

(2) Spectrophotometer, SPECTRONIC 21, Spectronics Instrument, USA

(3) Flow through cell (1 cm), Hellma, Germany

2.1.2 Chemicals

(1) Potassium permanganate, Extra pure, MERCK, Germany

(2) Sulfuric acid, 95-97% (w/w), GR Grade, MERCK, Germany

(3) L(+) Ascorbic acid, GR Grade, MERCK, Germany

(4) Ammonium Cerium (*IV*) sulphate, AR Grade, MERCK, Germany

(5) 1,10-Phenanthroline, AR Grade, MERCK, Germany

(6) Ferrous sulphate, AR Grade, MERCK, Germany

(7) Sodium hydroxide, AR Grade, AKZO NOBEL, Sweden

(8) Phenolphthalein, , AR Grade, MERCK, Germany

(9) Acetic acid (glacial), 100%, MERCK, Germany

(10) Ethanol, 95% (v/v), Lab-SCAN, Ireland

2.2 Samples

2.2.1 Samples for ascorbic acid determination

Samples investigated were vitamin C tablet samples (see APPENDIX G). Details of the samples are in Table 2.1.

Table 2.1 List of vitamin C tablet samples

Sample code	Concentration labeled (mg/tablet)	Note
A	1000	Effervescent
B	1000	Effervescent
C	500	Vitamin C
D	100	Sweetlets 100 mg
E	60	Chewable Zinc and Vitamin C Fruit Flavour Tablets
F	1000	Effervescent
G	100	Vitamin C
H	500	Sodium ascorbate
I	50	Vitamin C
J	100	Vitamin C

2.2.2 Sample for acetic acid determination

Samples investigated were locally commercial vinegar samples (see APPENDIX H). Details of the samples are in Table 2.2.

Table 2.2 List of locally commercial vinegar samples

Sample code	Concentration labeled (%w/v)	Note
1	5%	Distilled vinegar
2	5%	Distilled vinegar
3	5%	Distilled white vinegar
4	5%	Distilled vinegar
5	5%	Distilled vinegar
6	5%	Distilled vinegar
7	5%	Distilled vinegar
8	5%	Distilled vinegar
9	5%	Distilled vinegar
10	4%	Artificial vinegar
11	5%	Artificial vinegar

2.3 Preparation of solution

2.3.1 Preparation of reagents used in SIA system.

(a) Stock solution of 0.1 M potassium permanganate in 0.1 M sulfuric, 100 mL

The solution was prepared by dissolving 1.58 g of potassium permanganate in 0.1 M H_2SO_4 solution and made up into a 100 mL volume. This stock solution was then further diluted for appropriated concentration.

(b) Stock standard ascorbic acid solution, 2000 mg/L, 250 mL

This was prepared by dissolving 0.5000 g of ascorbic acid in deionized water in a 250 mL volumetric flask.

Working standard ascorbic acid solutions were freshly prepared by diluting the stock solution.

(c) Stock solution of 2 M sodium hydroxide, 250 mL

Stock solution was prepared by dissolving 20.00 g of sodium hydroxide in deionized water to a volume of 250 mL. This stock solution was then used to prepare various concentrations by suitable dilution.

(d) Stock solution of 0.2% (w/v) phenolphthalein in ethanol 50% (v/v) , 250 mL

The solution was prepared by dissolving 0.50 g of phenolphthalein in ethanol 50% (v/v) to make a volume of 250 mL.

Then this stock solution was used to prepare a various concentrations of phenolphthalein solutions.

(e) Stock standard acetic acid solution, 20% (w/v), 250 mL

A stock solution was prepared by weighting 50.00 g of gracial acetic acid into a 250 mL volumetric flask and diluted it to the mark with deionized water.

Working standard acetic acid solutions were freshly prepared by diluting the stock solution.

(f) Solution of vitamin C samples

By weighing 20 tablets of a vitamin C sample and grinding in a mortar, a portion of the ground sample was taken for the weight of a tablet and then it was dissolved with deionized water into 1 L.

2.3.2 Preparation of reagent used in the standard method

(a) 0.1 M ammonium cerium (IV) sulfate solution

The solution was prepared by weighting 65.00 g of ammonium cerium (IV) sulfate into mixing of 28.00 mL of concentrated sulfuric acid and 500 mL of deionized water. Then mixture solution was stirred until the solid had dissolved. The dissolved mixture was transferred to a 1 L volumetric flask and made up to the mark with deionized water.

(b) Ferroin indicator

It was prepared by dissolving 0.49 g of 1,10-phenanthroline hydrate (relative molecular mass = 198.1) in the solution of 0.02 M $FeSO_4$ then the dissolved mixture was transferred to a 100 mL volumetric flask and made up to the mark with 0.02 M $FeSO_4$ this solution is known as ferroin.

2.4 Determination of ascorbic acid by sequential injection spectrophotometric method

2.4.1 Schematic diagram

A schematic diagram of the sequential injection flow system is shown in Figure 2.1. All tubes in the system were 0.6 mm I.D. polytetrafluoroethylene (PTFE). The length of the holding coil (HC) and mixing coil (MC) were 200 and 100 cm respectively.

Double selection valves used in this system were seven-port valves, the first valve was interfaced to the second valve by passing port number one of the first selection valve and center port of the second selection valve. The second selection valve was connected to a mixing coil by port number two and a mixing coil was connected to a flow through cell in a spectrophotometer.

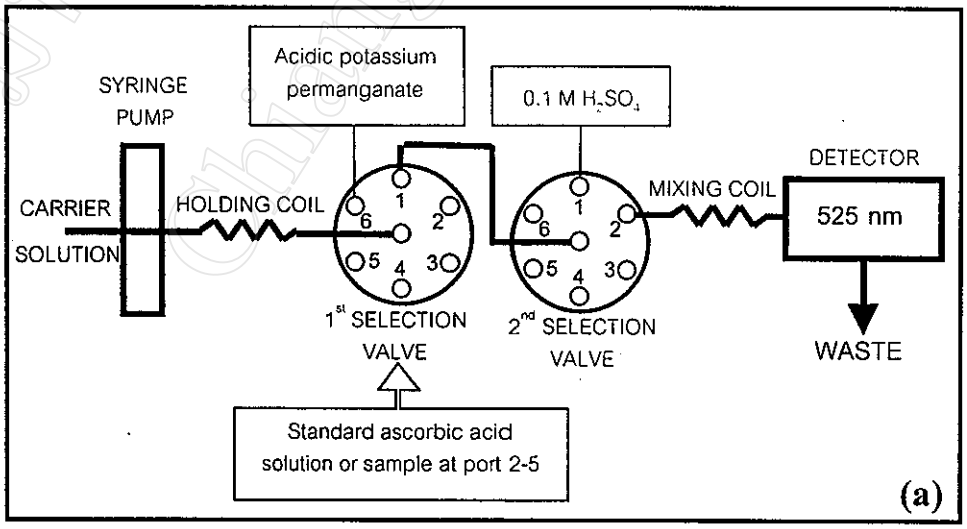

Figure 2.1(a) A schematic diagram of the SIA system for ascorbic acid determination.

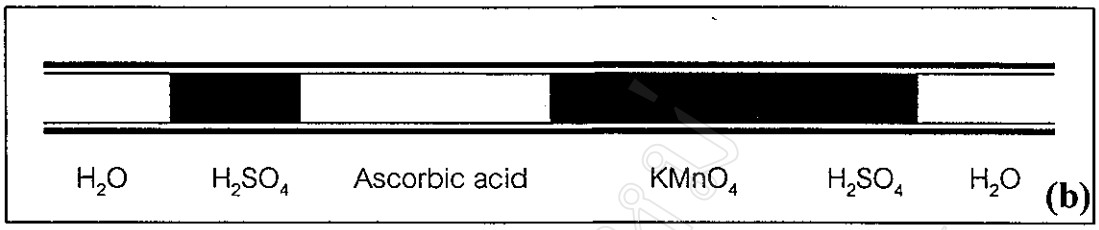

Figure 2.1 (b) A schematic diagram of the segment order for ascorbic acid determination.

2.4.2 Procedure

The ***"clean_system"*** program [APPENDIX C] was used as the beginning step for flushing and filling the lines with new solutions. Four measurement cycles consist of the following steps:

(1) Washing of the sample lines with a new sample solutions

In this step, a new sample or standard solution was aspirated to replace the solution existing previously in the line and to fill the sample lines.

(2) Aspiration of carrier solution

For beginning of a cycle, deionized water was aspirated to fill in the cylinder of syringe pump for flushing the solution in the line through the detector and filled in the line before starting next cycle.

(3) Aspiration of a desired sulfuric acid volume

Next step, 50 μL of 0.1 M H_2SO_4 was aspirated to keep in line between holding coil to the second selection valve to sandwich the zone of acidic potassium permanganate to prevent potassium permanganate zone from precipitation if not enough acidity.

(4) Aspiration of a desired ascorbic acid volume

In this step, the desired volume of sample or standard ascorbic acid solution was aspirated in the holding coil.

(5) Aspiration of desired acidic potassium permanganate solution volume

The desired volume of acidic potassium permanganate solution was aspirated in the holding coil.

(6) Detection of the excess acidic potassium permanganate colour

In the last step, the solution in holding coil was pumped through the mixing coil to detector and then to the waste. The concentration of excess acidic potassium permanganate was detected at 525 nm.

In all experiments, the measurement cycle was repeated three times for each concentration. The integrated area was plotted and printed out by using Microsoft Excel program. The AnalySIA program using in the measurement is shown in APPENDIX C.

2.4.3 Optimization for conditions for sequential injection determination of ascorbic acid

Preliminary conditions were set as in Table 2.3.

Table 2.3 Preliminary conditions for ascorbic acid determination

Parameter	Conditions
Aspiration volume of acidic potassium permanganate	100 μL
Aspiration volume of standard ascorbic acid solution	80 μL
Aspiration volume of 0.1 M sulfuric acid	50 μL
Speed of pump	150 μL /s

2.4.3.1 Effect of acidic potassium permanganate concentration

Using the system in Figure 2.1 and "***meas_as***" AnalySIA program, a blank and a series of standard ascorbic acid solutions were injected into the system using the conditions described in Table 2.3 but with different acidic potassium permanganate concentrations. The acidic potassium permanganate solution line was flushed and filled with the acidic potassium permanganate when changing a new concentration of acidic potassium permanganate. The results are

shown in Tables 2.4, 2.5 and Figures 2.2, 2.3. The results indicate that 2.5 x 10^{-3} M $KMnO_4$ in 0.1 M H_2SO_4 gave the promising linearity.

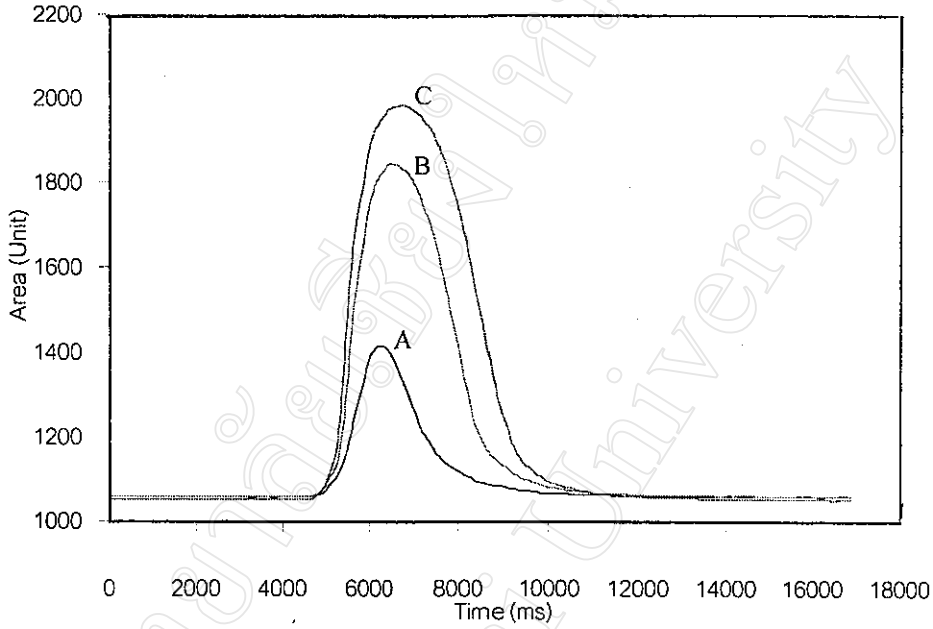

Figure 2.2 SIA peaks of different acidic potassium permanganate concentrations (a standard ascorbic acid concentration of 800 mg/L): (A) $1x10^{-3}$, (B) $2.5x10^{-3}$ and (C) $5x10^{-3}$ M of acidic potassium permanganate solution.

Table 2.4 Effect of acidic potassium permanganate concentration on peak area; mean of triplicate aspirations

Concentration of ascorbic acid (mg/L)	Area (Unit)		
	Concentration of acidic potassium permanganate (M)		
	1 x 10^{-3}	**2.5 x 10^{-3}**	**5 x 10^{-3}**
50	240.3	249.6	289.2
400	60.7	179.5	256.8
800	6.9	75.9	177.0
1200	4.5	24.3	84.4

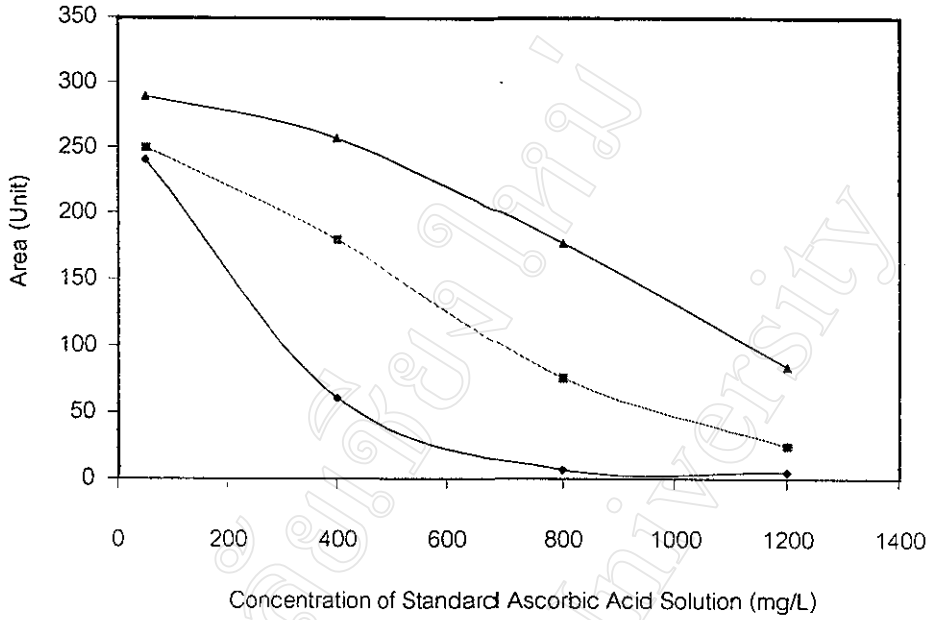

Figure 2.3 Effect of acidic potassium permanganate concentration on peak area: (◆)$1.0x10^{-3}$, (■) $2.5x10^{-3}$, (▲) $5.0x10^{-3}$ M of acidic potassium permanganate.

Table 2.5 Effect of acidic potassium permanganate concentration on linearity

Concentration of acidic potassium permanganate (M)	Linear equation	Correlation coefficient
$1.0x10^{-3}$	Y= -0.194X+197.1	0.7546
$2.5x10^{-3}$	Y= -0.202X+256.2	0.9841
$5.0x10^{-3}$	Y= -0.181X+312.9	0.9725

2.4.3.2 Effect of acidic potassium permanganate aspiration volume

Using the conditions described in 2.4.3.1, a blank and a series of standard ascorbic acid solutions were injected into the system with various aspiration volumes of acidic potassium permanganate. The results are shown in Tables 2.6, 2.7 and Figures 2.4, 2.5. An aspiration volume of 180 μL was chosen as giving the best linearity.

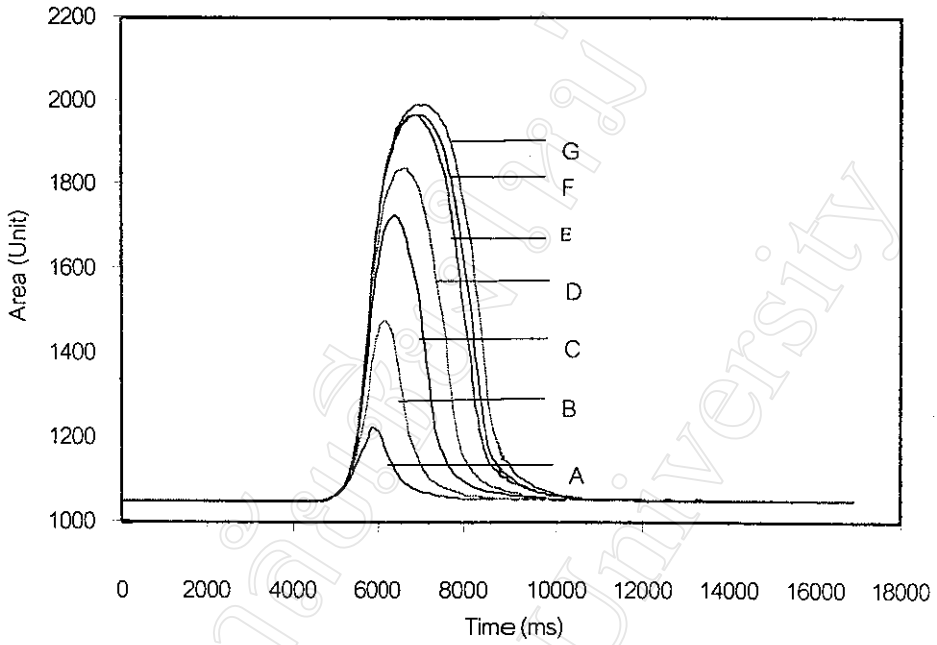

Figure 2.4 SIA peaks of different acidic potassium permanganate aspiration volumes by using a standard ascorbic acid concentration of 800 mg/L: (A) 100, (B) 120, (C) 140, (D) 160, (E) 180, (F) 200 and (G) 220 μL of acidic potassium permanganate solution.

Table 2.6 Effect of acidic potassium permanganate aspiration volume on peak area: mean of triplicate aspirations

Concentration of ascorbic acid (mg/L)	Area (Unit)						
	Aspiration volume of acidic potassium permanganate solution (μL)						
	100	**120**	**140**	**160**	**180**	**200**	**220**
0	182.0	219.8	253.3	277.9	294.3	306.0	320.6
400	60.9	112.9	155.9	193.7	224.3	234.7	250.8
800	16.3	40.1	81.0	117.2	159.7	171.5	185.3
1200	8.8	15.9	42.4	77.0	113.7	129.6	143.8

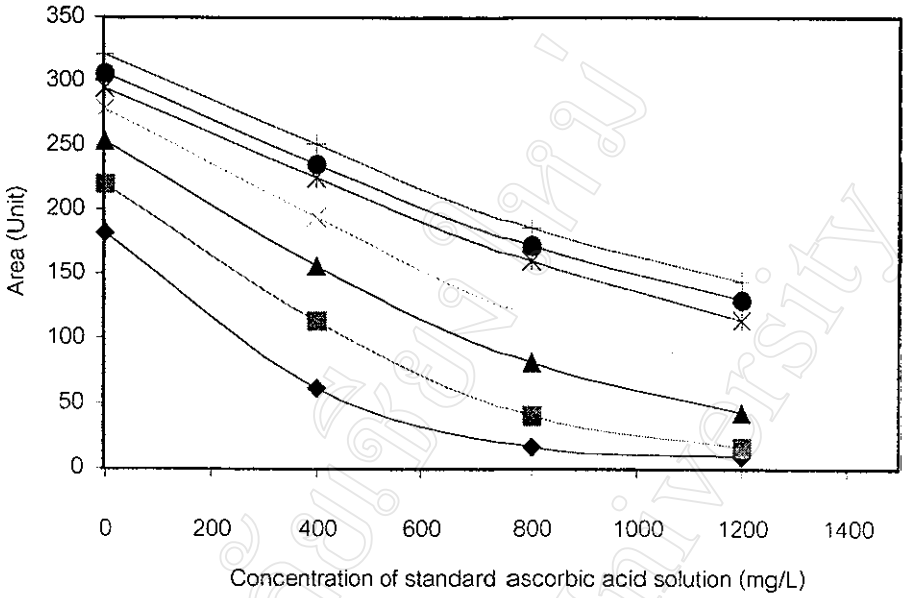

Figure 2.5 Effect of acidic potassium permanganate aspiration volume on peak area: (◆) 100, (■) 120, (▲) 140, (×) 160, (✳) 180,(●) 200 and (✙) 220 μL of acidic potassium permanganate solution.

Table 2.7 Effect of acidic potassium permanganate aspiration volume on linearity

Aspiration volume of acidic potassium permanganate (μL)	Linear equation	Correlation coefficient
100	y = -0.141x + 151.6	0.8283
120	y = -0.171x + 199.9	0.9317
140	y = -0.177x + 239.3	0.9663
160	y = -0.170x + 268.3	0.9776
180	y = -0.152x + 289.0	0.9917
200	y = -0.148x + 299.3	0.9873
220	y = -0.149x + 314.5	0.9879

2.4.3.3 Effect of ascorbic acid aspiration volume

Using the conditions described in 2.4.3.2, a blank and a series of standard ascorbic acid solutions were injected into the system but various aspiration volumes of ascorbic acid were changed in series of standard ascorbic acid solution. The results are shown in Tables 2.8, 2.9 and Figures 2.6, 2.7, so 80 μL of the standard ascorbic acid solution was chosen to give a good linearity.

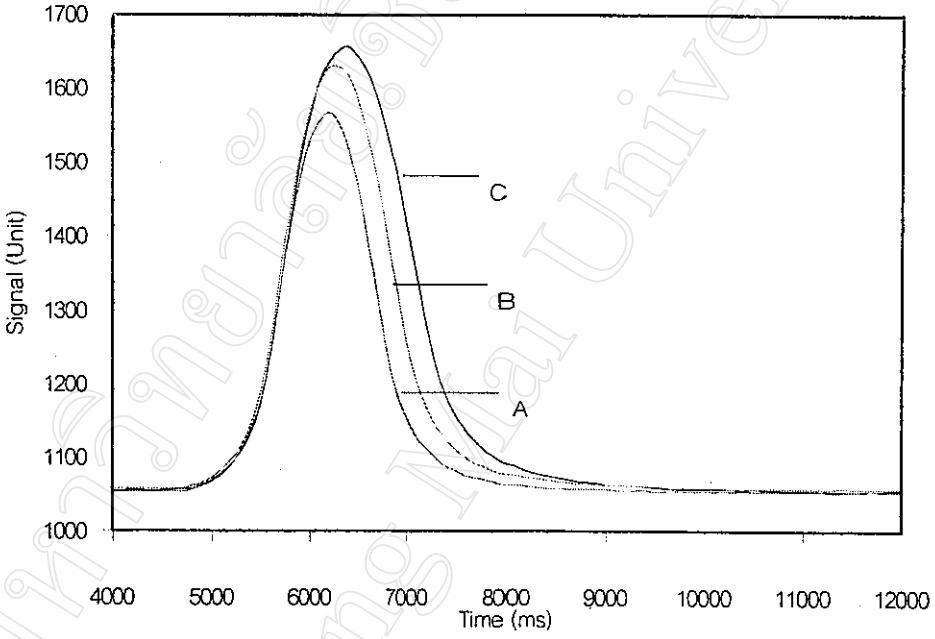

Figure 2.6 SIA peaks of different ascorbic acid aspiration volumes: (A) 120, (B) 100 and (C) 80 μL of standard ascorbic acid (800 mg/L).

Table 2.8 Effect of ascorbic acid aspiration volume on peak area; mean of triplicate aspirations

Concentration of ascorbic acid (mg/L)	Area (Unit)		
	Aspiration volume of standard ascorbic acid solution (μL)		
	80	**100**	**120**
0	293.2	291.6	295.6
400	220.8	209.7	204.3
800	161.6	145.7	141.7
1200	117.9	106.3	101.8

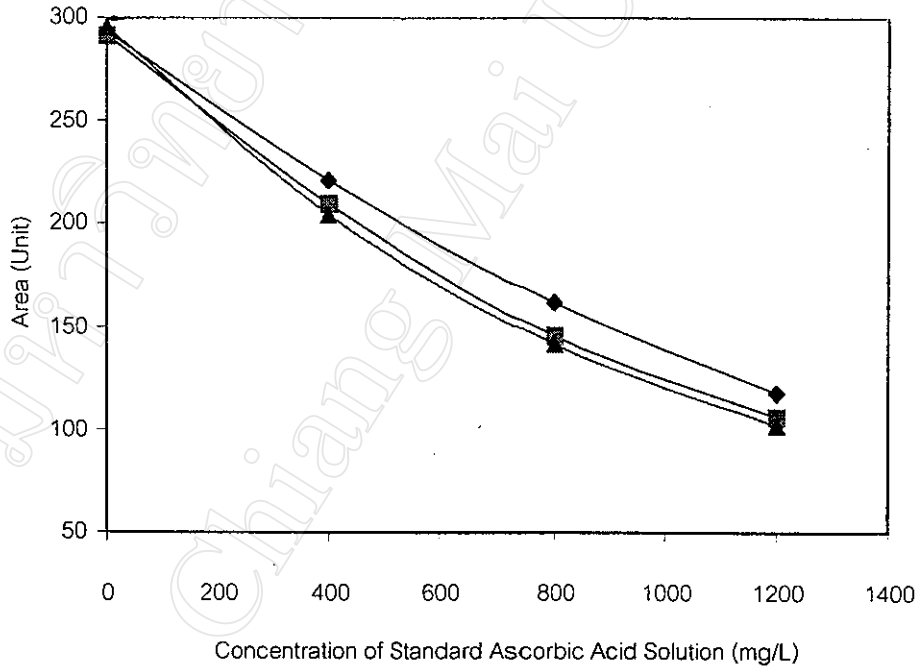

Figure 2.7 SIA peaks for different ascorbic acid aspiration volumes at a standard ascorbic acid concentration of 800 mg/L: (◆) 80, (■) 100 and (▲) 120 μL of a standard ascorbic acid solution.

Table 2.9 Effect of acidic potassium permanganate concentration on linearity

Aspiration volume(μL)	Linear equation	Correlation coefficient
80	y = -0.146x + 286.1	0.9882
100	y = -0.155x + 281.3	0.9770
120	y = -0.161x + 282.4	0.9691

2.4.3.4 Effect of pump speed (flow rate)

The effect of pump speed to drive the mixed solution through detector was studied. Using the conditions described in 2.4.3.3, in this experiment, a chart recorder was used to record the detector signal and peak height was used for a calibration graph. A blank and a series of standard ascorbic acid solutions were injected into the system. The results are shown in Tables 2.10, 2.11 and Figure 2.8. All the calibration graphs studied were linear for all the pump speeds, if pump speed was low the longer time consuming was observed and slope was higher than a higher pump speed. So a pump speed of 150 μL/s was chosen as giving high slope, good linearity and suitable time consuming.

Table 2.10 Effect of pump speed on peak height; mean of triplicate aspirations

Concentration of ascorbic acid (mg/L)	Peak Height (cm.)				
	Speed of pump (μL/s)				
	50	**100**	**150**	**200**	**250**
0	23.6	23.6	23.6	23.9	23.9
500	19.8	19.7	19.8	20.4	20.4
1000	15.2	15.7	16.1	17.1	17.0
1500	11.2	13.0	13.2	14.5	14.4

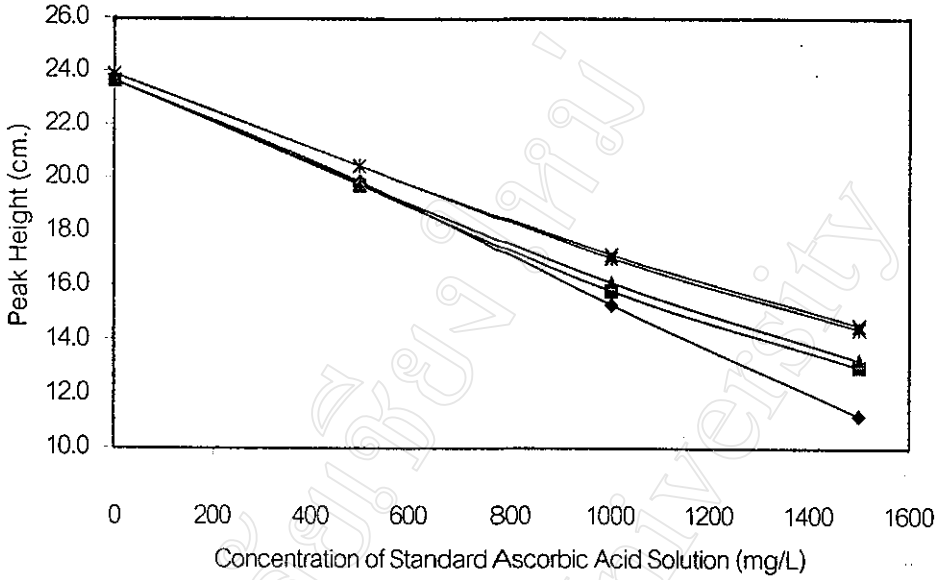

Figure 2.8 Effect of pump speed on peak height; mean of triplicate aspirations: (◆) 50, (■) 100, (▲) 150, (×) 200 and (✳) 250 μL/s of pump speed.

Table 2.11 Effect of pump speed on linearity

Speed of pump (μL/s)	Linear equation	Correlation coefficient
50	y = 0.0084x + 23.7	0.9989
100	y = 0.0072x + 23.4	0.9940
150	y = 0.0070x + 23.4	0.9954
200	y = 0.0063x + 23.7	0.9960
250	y = 0.0064x + 23.7	0.9958

2.4.3.5 Summary of the conditions used

The SIA system schematic was used as depicted in Figure 2.1 with a procedure described in 2.4.2 and the conditions are summarized in Table 2.12.

Table 2.12 The SIA conditions for ascorbic acid determination

Parameter	Conditions
Aspiration volume of $2.5x10^{-3}$ M acidic potassium permanganate solution	100 µL
Aspiration volume of standard ascorbic acid solution	80 µL
Aspiration volume of 0.1 M sulfuric acid solution	50 µL
Speed of pump	150 µL/s

2.4.3.6 Calibration range

Using the SIA system described in 2.4.3.5. The calibration range was studied. The results are shown in Table 2.13 and Figures 2.9 and 2.10. A linear range up to 1200 mg/L and the sample throughput of 60 h^{-1} were obtained. The calibration range was in the range of sample concentrations, which are commonly found.

Table 2.13 Calibration range of standard ascorbic acid

Ascorbic acid (mg/L)	Area (Unit)	Ascorbic acid (mg/L)	Area (Unit)
0	238.6	700	156.4
40	233.8	800	144.1
100	227.7	900	129.5
200	218.6	1000	123.2
300	207.5	1100	104.7
400	202.5	1200	98.7
500	188.9	1400	84.2
600	171.8	1600	70.8

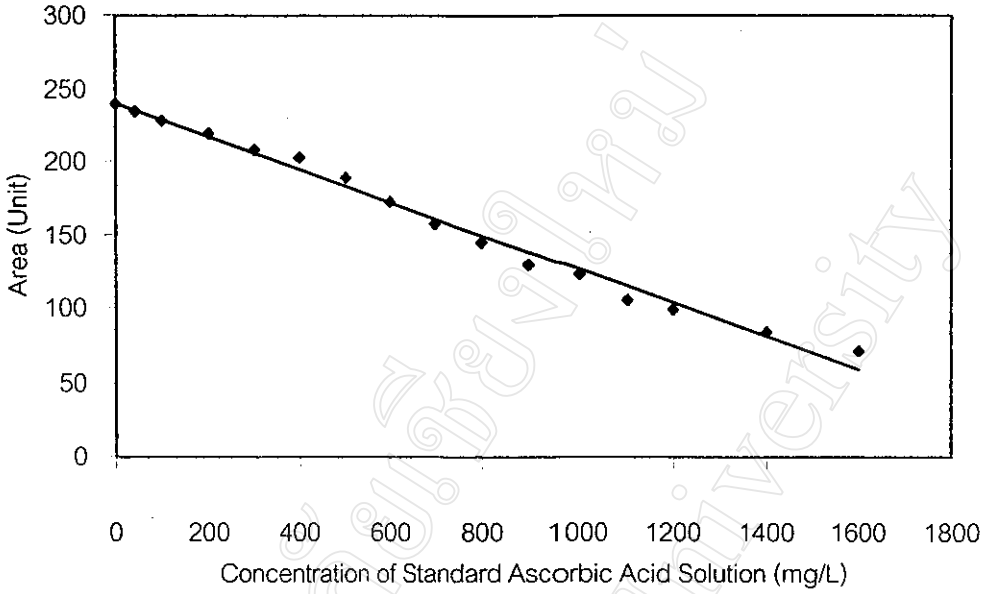

Figure 2.9 Calibration range of standard ascorbic acid (0-1600 mg/L):
$y = -0.113x + 239.2$, $R^2 = 0.9890$.

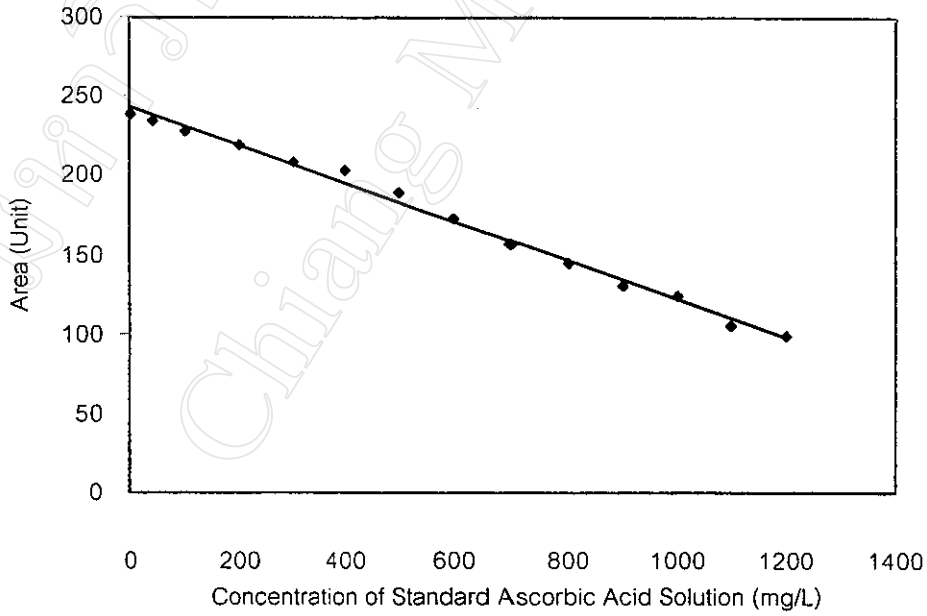

Figure 2.10 Calibration range of standard ascorbic acid (0-1200 mg/L):
$Y = -0.121 x + 242.3$, $R^2 = 0.9933$.

2.4.3.7 Precision

Using the SIA conditions described in 2.4.3.5, precision by repeating aspiration of 400 mg/L and 1200 mg/L of standard ascorbic acid solutions for 11 replicates were studied. The results are shown in Table 2.14 and Figures 2.11, 2.12.

The relative standard deviations (RSD) are 2.9 % for 400 mg/L and 1.8 % for 1200 mg/L.

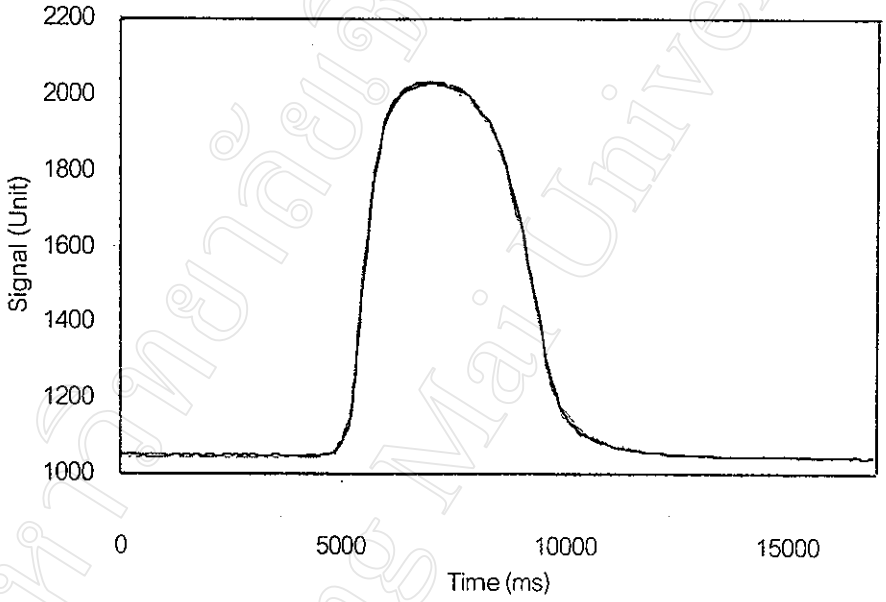

Figure 2.11 SIA peaks of 11 replicate aspirations of 400 mg/L standard ascorbic acid solution.

Table 2.14 A precision study

Number of aspiration	Peak area (Unit)	
	Concentration of standard ascorbic acid solution	
	400 mg/L	1200 mg/L
1	264.4	142.5
2	267.5	140.9
3	266.4	137.9
4	263.8	140.0
5	260.8	138.1
6	263.1	143.7
7	264.3	139.0
8	268.9	143.7
9	267.8	144.1
10	265.3	137.6
11	241.3	143.0

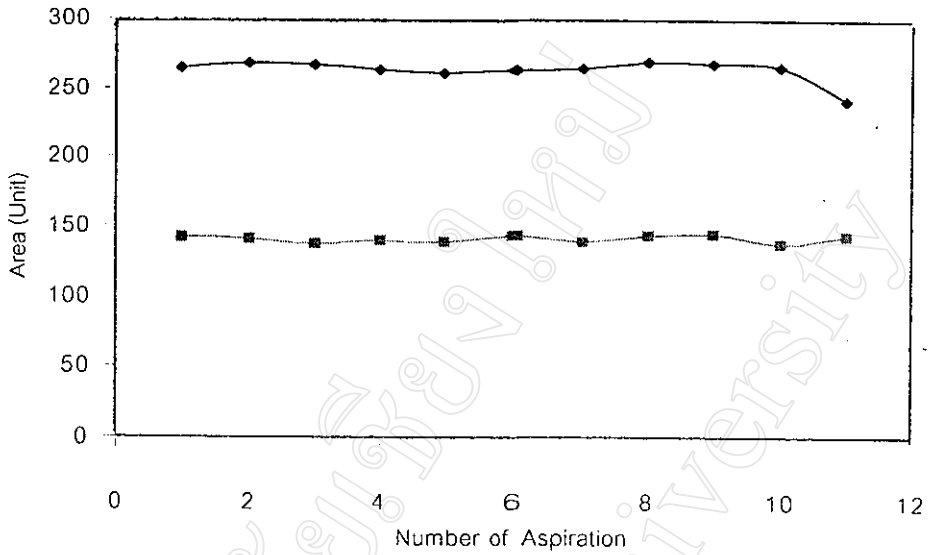

Figure 2.12 A precision study: (◆) 400 mg/L and (■) 1200 mg/L of standard ascorbic acid solutions.

2.4.3.8 Determination of ascorbic acid in vitamin C tablet sample

The optimized system was applied to the determination of ascorbic acid in locally commercial vitamin C tablet samples. The preparation of sample solution was described in 2.3.1(f). A titrimetric method [APPENDIX A] for reference purpose was also carried out. The results are shown in Tables 2.15, 2.16, 2.17 and Figures 2.13, 2.14. The comparison of the results obtained by the SIA method was evaluated by t-test [23]. The calculated t-test value was 0.67. The critical value of t-test was 2.26 (9 degrees of freedoms) at confidence interval of 95% and since the calculated value of t-test was less than the critical value. The results from the two recommended methods were not significantly different (with a confidence interval of 95%).

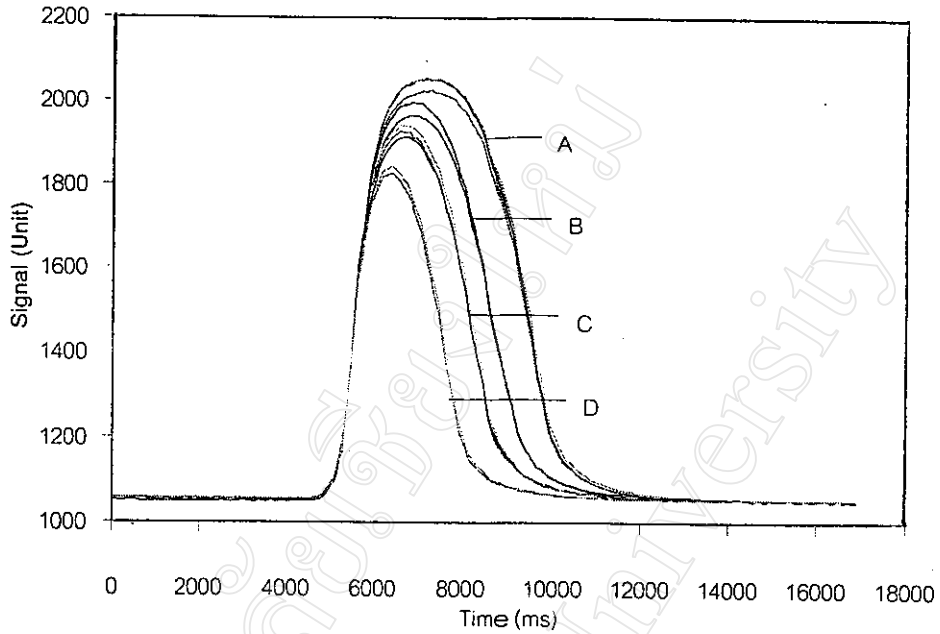

Figure 2.13 SIA peaks for calibration graph of ascorbic acid determination: (A) blank, (B) 400, (C) 800 and (D) 1200 mg/L of standard ascorbic acid.

Table 2.15 Calibration graph of ascorbic acid determination

Concentration of ascorbic acid (mg/L)	Area (Unit)
0	320.6
400	262.4
800	203.2
1200	163.6

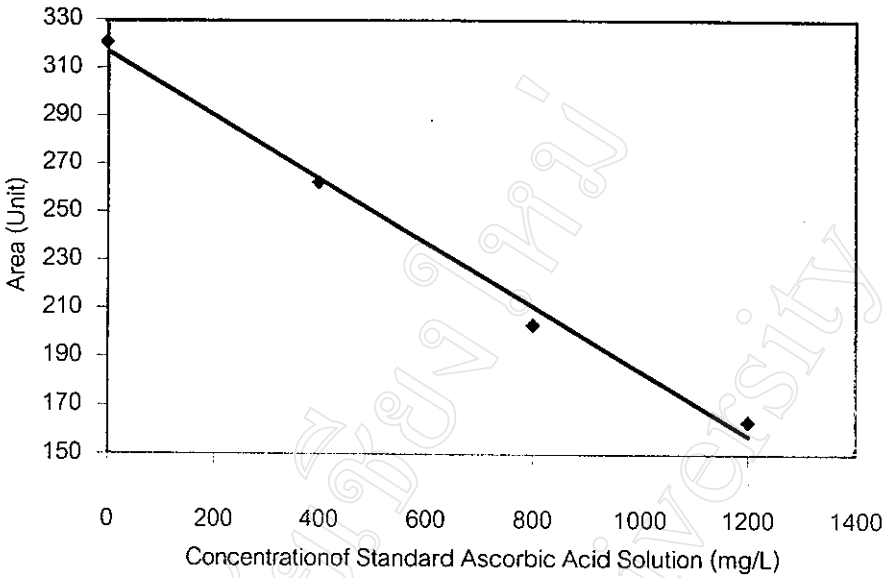

Figure 2.14 Calibration graph of ascorbic acid determination: y = -0.133x + 317.0, $R^2 = 0.9924$.

Table 2.16 Determination of ascorbic acid in vitamin C tablet samples

Sample code	Labeled amount (mg/tablet)	Ascorbic acid founded (mg/L)			
		Titrimetric method		SIA method	
		mg/tablet	%label	mg/tablet	%label
A	1000	1022	102	1014	101
B	1000	1013	101	892	89
C	500	477	95	520	104
D	100	101	101	101	101
E	60	69	115	72	120
F	1000	957	96	951	95
G	100	95	95	104	104
H	500	459	92	512	102
I	50	49	98	48	96
J	100	103	103	103	103

2.4.3.9 Recovery study

Using the optimized conditions in section 2.4.3.5, a series of sample with standards added was prepared by pipetting 0, 20, and 40 mL of 2000 mg/L of standard ascorbic acid solution into each volumetric flask (100 mL) and to each was added with 50 mL of the sample solution (sample code D). Finally each flask was diluted to the mark with deionized water. Thus 0, 400 and 800 mg/L of standard ascorbic acid added in sample were obtained. Each solution was aspirated into the SIA system. The results are shown in Figures 2.15, 2.16, 2.17 and Tables 2.18, 2.19.

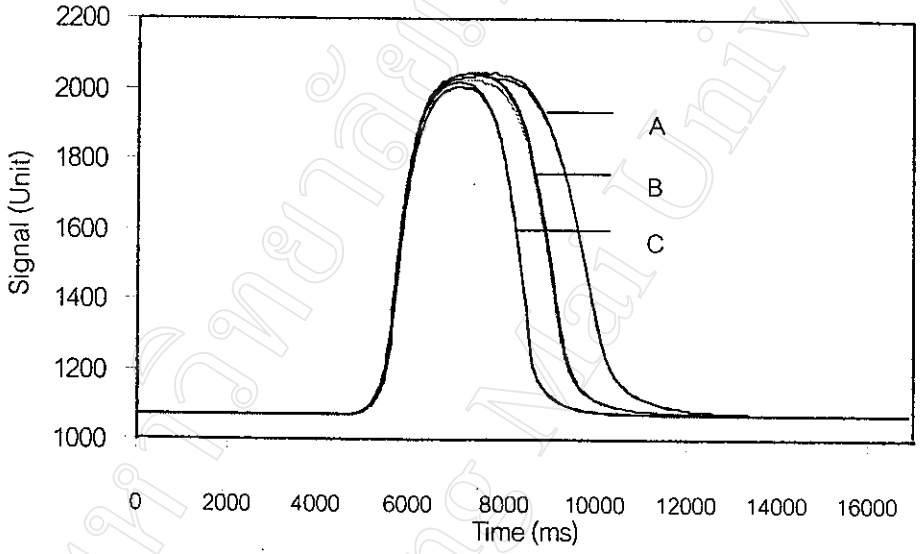

Figure 2.15 SIA peaks of sample added with ascorbic acid standards: (A) Blank, (B) 400 and (C) 800 mg/L.

Table 2.17 Calibration graph of SIA ascorbic acid

Ascorbic acid (mg/L)	Area (Unit)	Area after blank* subtraction
Blank (0)	318.2	0.0
400	256.2	62.0
800	196.6	121.6
1200	157.2	161.0

(*Blank value = 318.2 unit)

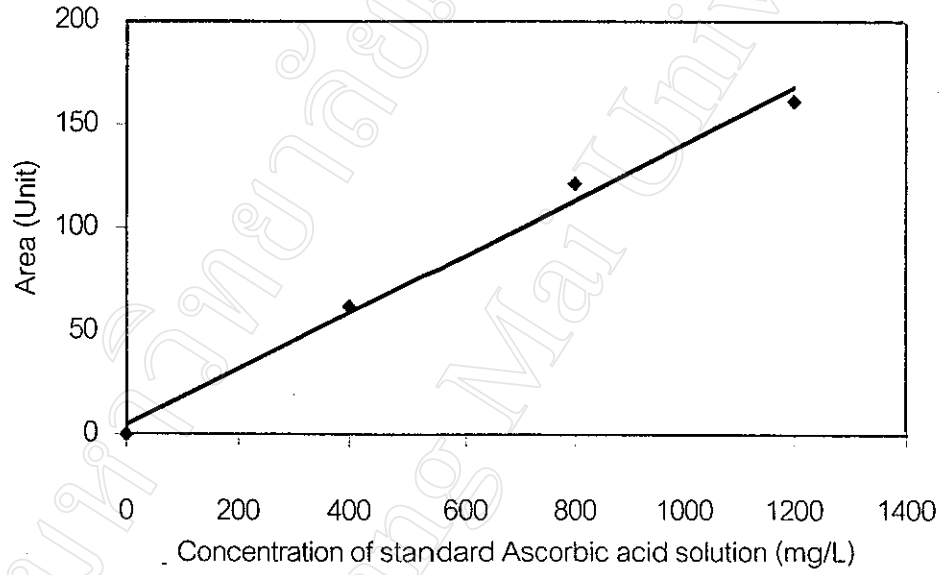

Figure 2.16 Calibration graph ($y = 0.136x + 4.7$, $R^2 = 0.9903$).

Table 2.18 Recovery study

Ascorbic acid added (mg/L)	Area (Unit)	Area after blank* subtracted (Unit)
0	297.4	20.8
400	239.0	79.3
800	183.9	134.3

(*Blank value = 318.2 unit)

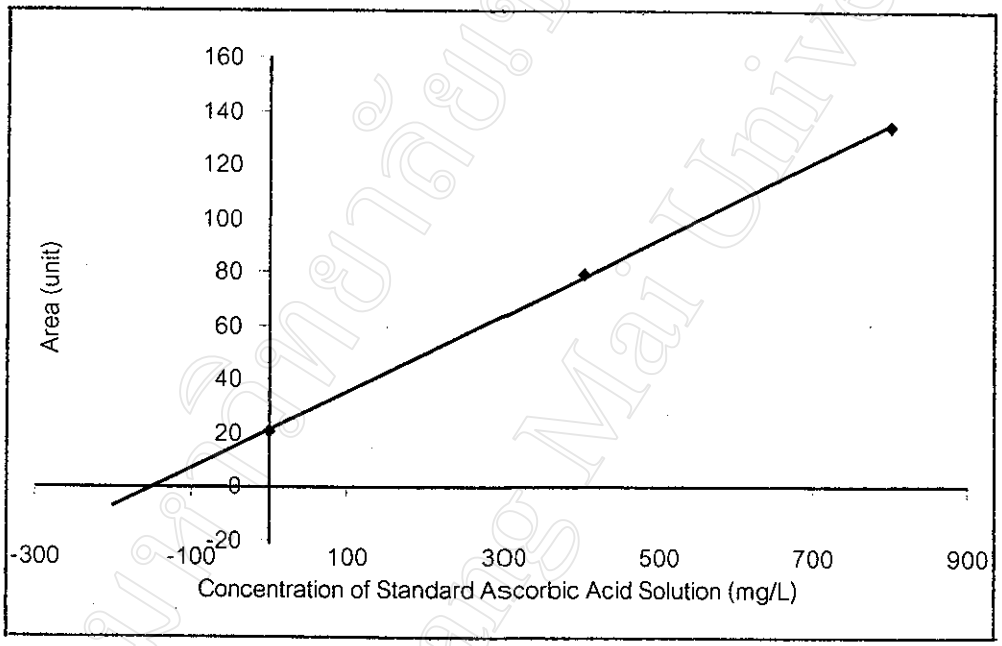

Figure 2.17 Standard addition curve of SIA ascorbic acid determination, used (blank-area) data plotted (data of the sample code D): $y = 0.142x + 21.4$ $R^2 = 0.9997$

Table 2.19 Study of percent recovery for SIA ascorbic acid determination

Ascorbic acid added (mg/L) (A)	Area (Unit)	Concentration of ascorbic acid + sample from calibration graph (mg/L) (B)	Recovery of standard added (mg/L) (C)	Percent recovery of standard added (mg/L) (D)
0	20.8	118.3	-	-
400	79.3	549.2	431	108
800	134.3	955.1	837	105

C = B-A; D= C/Ax100

Note* Concentration of the sample was found to be 151 mg/L (Figure 2.17).

2.5 Determination of acetic acid by sequential injection spectrophotometric method

2.5.1 Schematic Diagram

A schematic diagram of the sequential injection flow system is shown in Figure 2.18. The instrument used in this experiment was similar to that described in 2.4.1.

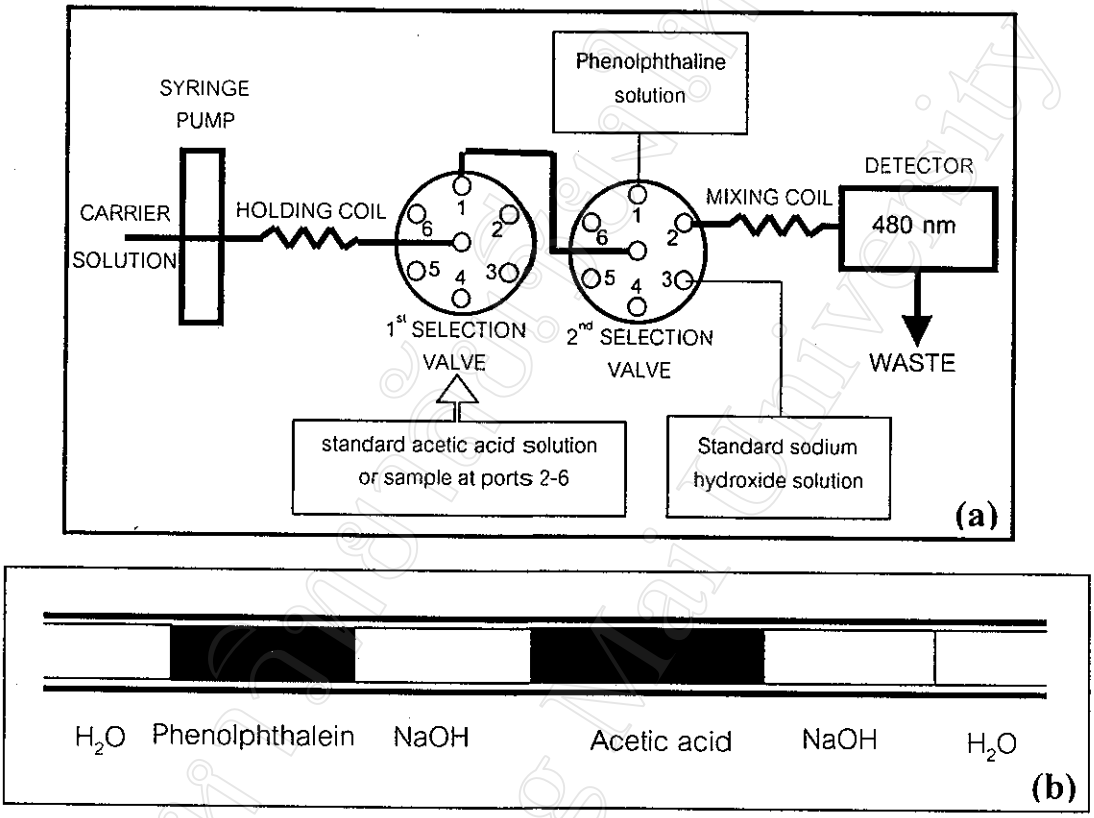

Figure 2.18 (a) A schematic diagram of the SIA system for acetic acid determination.
(b) A schematic diagram of the segment order for acetic acid determination.

2.5.2 Procedure

The ***"clean_system"*** program [APPENDIX C] was used as the beginning step for flushing and filling the lines with new solutions. Four measurement cycles consist of the following steps:

(1) Washing of the sample lines with the new of sample solutions

In this step, a new sample or a standard solution was aspirated to replace the previous solution and to fill the sample lines.

(2) Aspiration of desired phenolphthalein solution volume

For beginning of a cycle, the desired volume of phenolphthalein solution was aspirated in the holding coil.

(3) Aspiration of desired sodium hydroxide solution volume

A next step, the desired volume of sodium hydroxide solution was aspirated in the holding coil.

(4) Aspiration of desired acetic acid solution volume

In this step, the desired volume of sample or standard acetic acid solution was aspirated in holding.

(5) Aspiration of carrier solution

After that, deionized water was aspirated in the cylinder of syringe pump to drive mixed solution in the line through the detector and filled in the line before starting next cycle.

(6) Detection of the residue sodium hydroxide color

Finally the solution in holding coil was pumped through the mixing coil to detector and then to the waste. The concentration of excess sodium hydroxide solution was detected at 480 nm [23].

In all experiment, the measurement cycle was repeated three times for each concentration. The integrated area was plotted and printed out by using excel program. The AnalySIA program using in the measurement was shown in appendix E.

2.5.3 Optimization of sequential injection determination of acetic acid

Preliminary conditions were set in Table 2.20.

Table 2.20 Preliminary conditions for acetic acid determination.

Parameter	Conditions
Aspiration volume of 0.2 M sodium hydroxide solution	60 μL
Aspiration volume of standard acetic acid solution	50 μL
Aspiration volume of 1 x 10^{-2} % (w/v) phenolphthalein solution	50 μL
Speed of pump	150 μL/s

2.5.3.1 Effect of sodium hydroxide concentration

Using the system in figure 2.12 and "***meas_aa***" AnalySIA program, a blank and a series of standard acetic acid solution were injected into the system using the conditions described in Table 2.20 but different sodium hydroxide concentrations were used. The sodium hydroxide solution line was washed and filled with the new sodium hydroxide solution when concentration of sodium hydroxide solution was changed. The results are shown in Tables 2.21, 2.22 and Figures 2.19, 2.20. The results indicated that 0.8 M sodium hydroxide solution give the highest slope and best linearity.

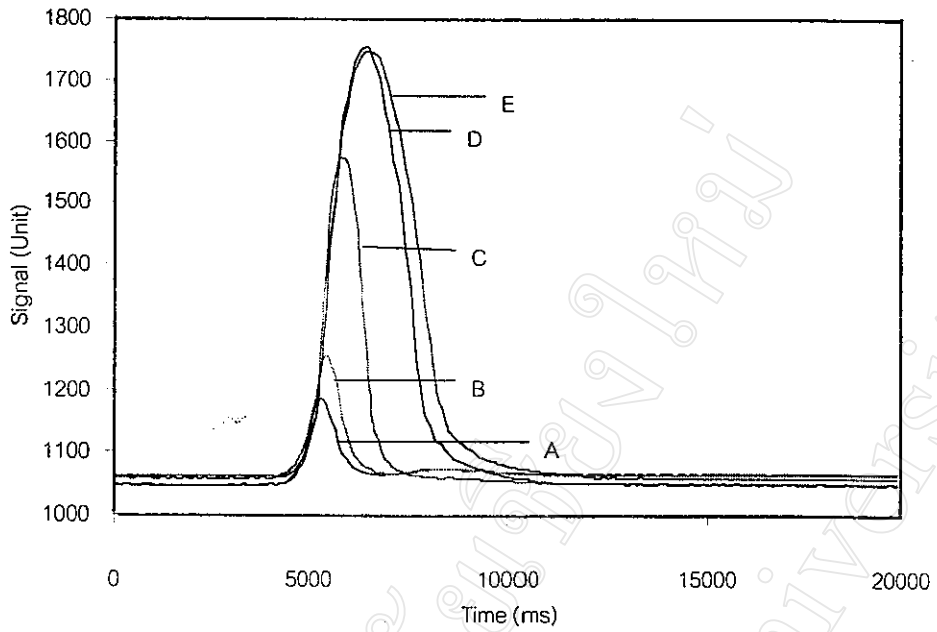

Figure 2.19 SIA peaks for different sodium hydroxide concentrations (a standard acetic acid concentration of 4%(w/v)): (A) 0.2, (B) 0.4, (C) 0.6, (D) 0.8 and 1.0 M of sodium hydroxide.

Table 2.21 Effect of sodium hydroxide concentration on peak area; mean of triplicate aspirations

Concentration of acetic acid (%w/v)	Area (Unit)				
	Concentration of sodium hydroxide (M)				
	0.2	**0.4**	**0.6**	**0.8**	**1.0**
0.0	152.0	154.6	149.6	142.7	133.7
2.1	55.3	84.2	128.8	139.0	135.3
4.2	11.3	14.5	44.6	100.9	111.5
6.3	7.8	9.8	14.4	53.2	67.3
8.4	7.1	6.3	9.6	21.4	29.3

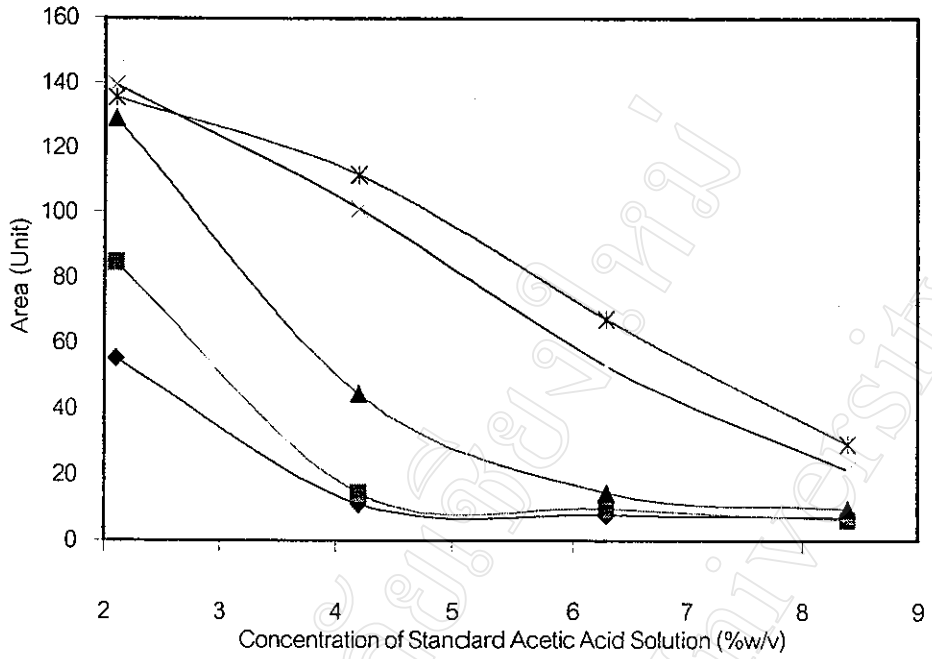

Figure 2.20 Effect of sodium hydroxide concentration on peak area: (◆) 0.2, (■) 0.4, (▲) 0.6, (×) 0.8 and (✳) 1.0 M of sodium hydroxide solution.

Table 2.22 Effect of sodium hydroxide concentration on linearity

Concentration of sodium hydroxide (M)	Linear equation	Correlation coefficient
0.2	y = -7.056x + 57.4	0.6696
0.4	y = -11.352x + 88.3	0.6864
0.6	y = -18.457x + 146.3	0.8225
0.8	y = -19.071x + 178.8	0.9947
1.0	y = -17.253x + 176.5	0.9871

2.5.3.2 Effect of sodium hydroxide aspiration volume

Using the condition describe in 2.5.3.1, a blank and a series of standard acetic acid solution were injected into the system with various aspiration volume of sodium hydroxide. The results are shown in Tables 2.23, 2.24 and Figures 2.21, 2.22. The aspiration volume of 70 μL was chosen as giving good linearity.

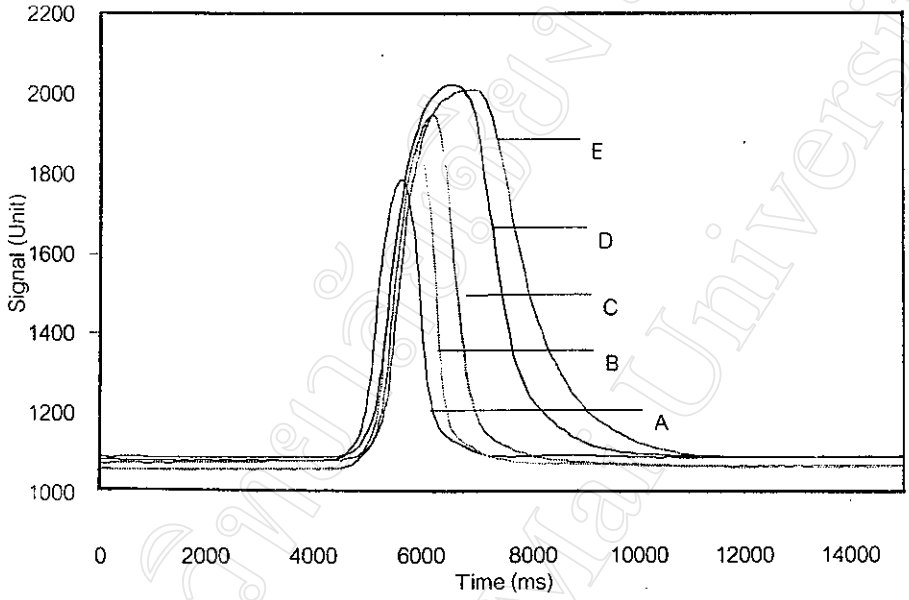

Figure 2.21 SIA peaks for different aspiration volume of sodium hydroxide solutions (a standard acetic acid concentration of 4%(w/v)): (A) 50, (B) 60, (C) 70, (D) 80 and (E) 90 μL of 0.8 M sodium hydroxide solution.

Table 2.23 Effect of aspiration volume of sodium hydroxide solution on peak area; mean of triplicate aspirations

Concentration of acetic acid (%w/v)	Area (Unit)				
	Aspiration volume of sodium hydroxide solution (μL)				
	50	**60**	**70**	**80**	**90**
2	308.6	303.2	276.2	285.8	282.4
4	204.4	201.8	210.2	296.4	318.9
6	125.0	117.9	140.1	198.3	260.2
8	80.6	63.6	84.8	143.7	156.6

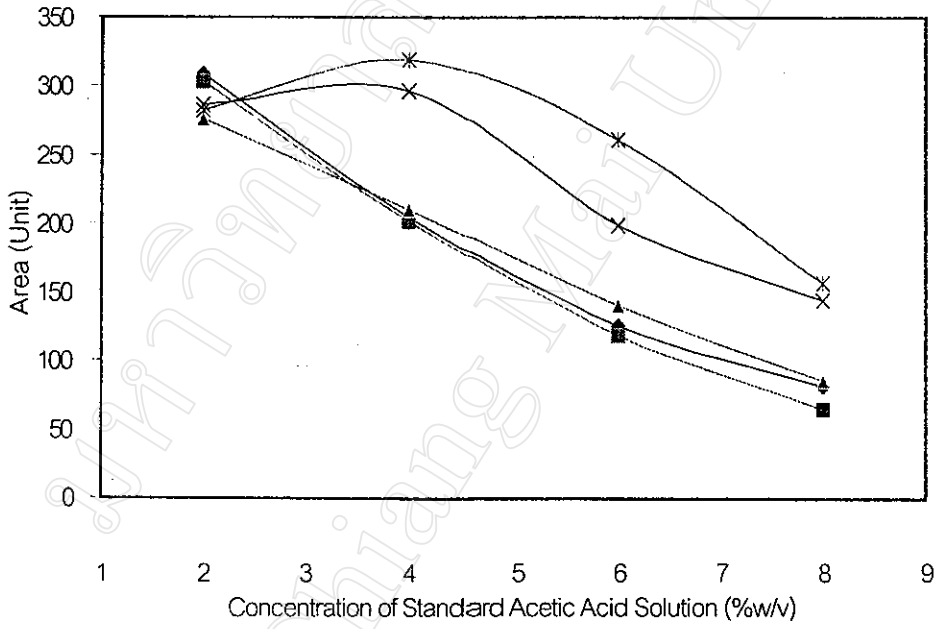

Figure 2.22 Effect of aspiration volume of sodium hydroxide solution on peak area : (◆) 50, (■) 60, (▲) 70, (×) 80 and (✳) 90 μL of sodium hydroxide solution.

Table 2.24 Effect of aspiration volume of sodium hydroxide solution on linearity

Aspiration volume of sodium hydroxide (μL)	Linear equation	Correlation coefficient
50	y = -38.173x + 370.5	0.9701
60	y = -40.124x + 372.3	0.9828
70	y = -32.204x + 338.8	0.9978
80	y = -26.230x + 362.2	0.8610
90	y = -21.791x + 363.5	0.6536

2.5.3.3 Effect of phenolphthalein concentration

Using the conditions described in 2.5.3.2, a blank and a series of standard acetic acid solution were injected into the system but different phenolphthalein concentrations were used. The phenolphthalein solution line was washed and filled with new phenolphthalein solution when concentration of phenolphthalein solution was changed. The results are shown in Tables 2.25, 2.26 and Figures 2.23, 2.24. The results indicated both of high slope and good linearity were obtained from a 2 x 10^{-2} % (w/v) phenolphthalein solution.

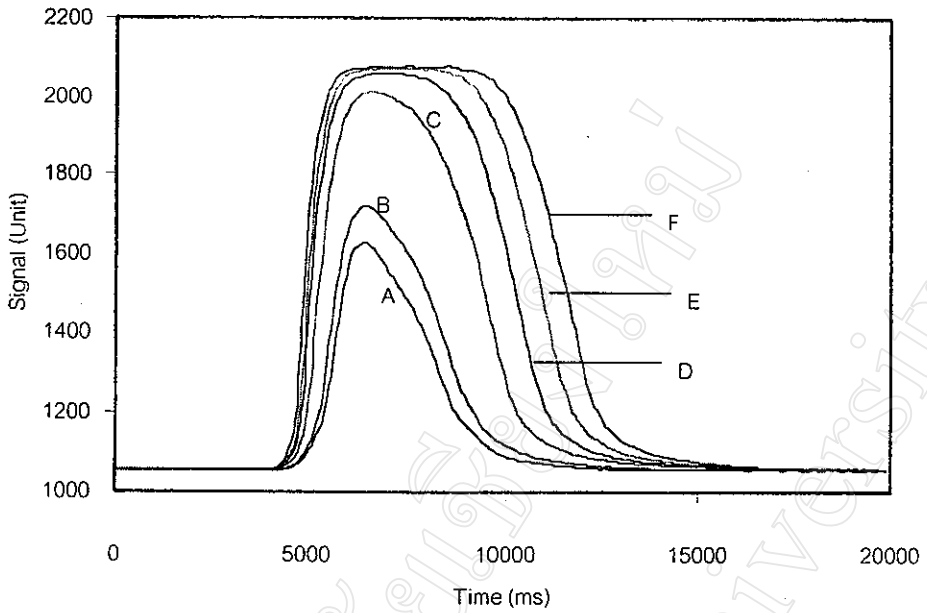

Figure 2.23 SIA peaks of different phenolphthalein concentrations (a standard acetic acid concentration of 4%(w/v)): (A) 8 x 10^{-3}, (B) 1 x 10^{-2}, (C) 2 x 10^{-2}, (D) 4 x 10^{-2}, (E) 6 x 10^{-2} and (F) 8 x 10^{-2} %(w/v) of phenolphthalein.

Table 2.25 Effect of phenolphthalein concentration on peak area; mean of triplicate aspirations.

Concentration of Acetic acid (%w/v)	Area (Unit)					
	Concentration of phenolphthalein (%w/v)					
	8 x 10^{-3}	**1 x 10^{-2}**	**2 x 10^{-2}**	**4 x 10^{-2}**	**6 x 10^{-2}**	**8 x 10^{-2}**
2	105.9	140.8	259.3	343.0	391.4	434.8
4	75.5	99.7	178.7	225.4	257.7	293.4
6	31.2	49.0	89.7	126.2	144.8	165.2
8	12.2	17.2	42.8	69.2	82.4	99.3

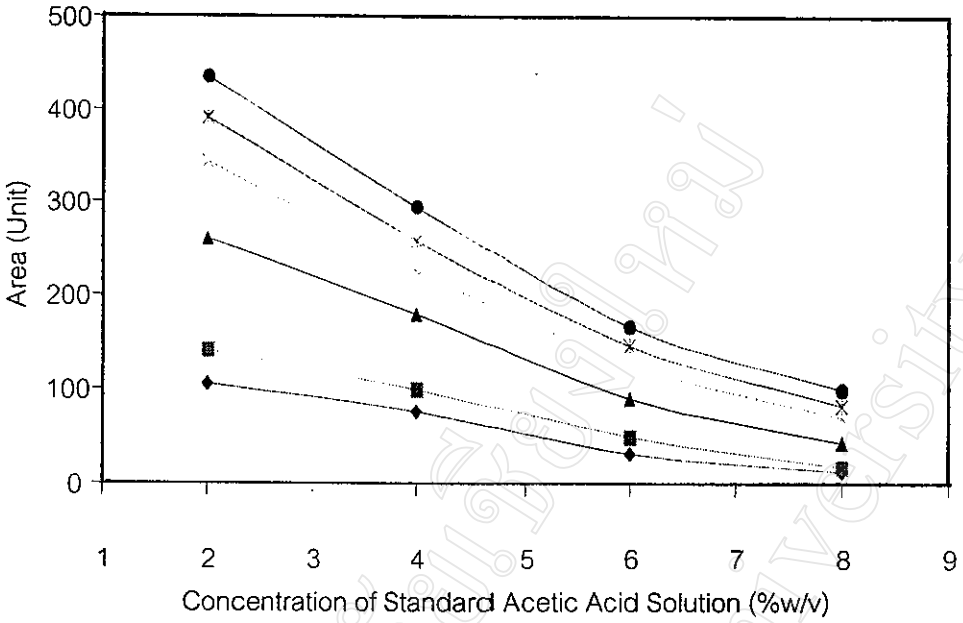

Figure2.24 Effect of phenolphthalein concentration on peak area: (◆) 8 x 10^{-3}, (■) 1 x 10^{-2}, (▲) 2 x 10^{-2}, (×) 4 x 10^{-2}, (✳) 6 x 10^{-2} and (●) 8 x 10^{-2} %(w/v) of phenolphthalein.

Table 2.26 Effect of phenolphthalein concentration on linearity

Concentration of phenolphthalein (%w/v)	Linear equation	Correlation coefficient
8 x 10^{-3}	y = -15.501x + 137.6	0.9799
1 x 10^{-2}	y = -20.077x + 182.1	0.9930
2 x 10^{-2}	y = -35.170x + 327.3	0.9851
4 x 10^{-2}	y = -43.833x + 421.1	0.9781
6 x 10^{-2}	y = -49.521x + 479.1	0.9762
8 x 10^{-2}	y = -54.024x + 531.8	0.9765

2.5.3.4 Effect of phenolphthalein aspiration volume

Using the conditions described in 2.5.3.3, a blank and a series of standard acetic acid solution were injected into the system with various aspiration volume of phenolphthalein solution. The results are shown in Tables 2.27, 2.28 and Figures 2.25, 2.26, so 70 μL of phenolphthalein solution was chosen to give a good linearity and height slope.

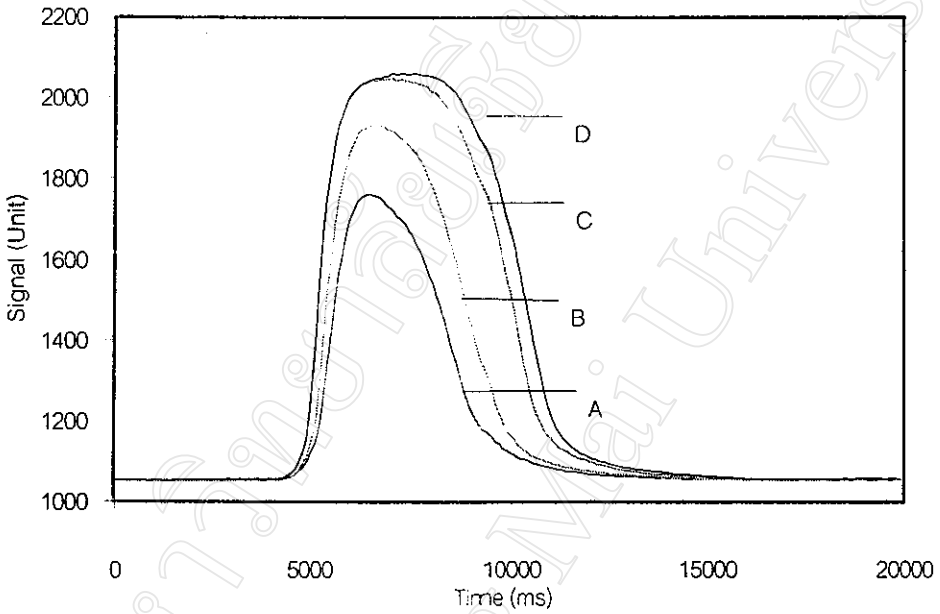

Figure 2.25 SIA peaks of different aspiration volume of phenolphthalein (a standard acetic acid concentration of 4%(w/v)): (A) 50, (B) 60, (C) 70 and (D) 80 μL of 2×10^{-2} %(w/v) phenolphthalein.

Table 2.27 Effect of aspiration volume of phenolphthalein on peak area; mean of triplicate aspirations

Concentration of Acetic acid (%w/v)	Area (Unit)			
	Volume of 2 x 10^{-2} % phenolphthalein (μL)			
	50	**60**	**70**	**80**
2.1	259.3	210.1	306.2	335.4
4.2	178.7	180.3	207.8	217.7
6.3	89.7	103.2	108.4	109.8
8.4	42.8	51.2	51.7	54.5

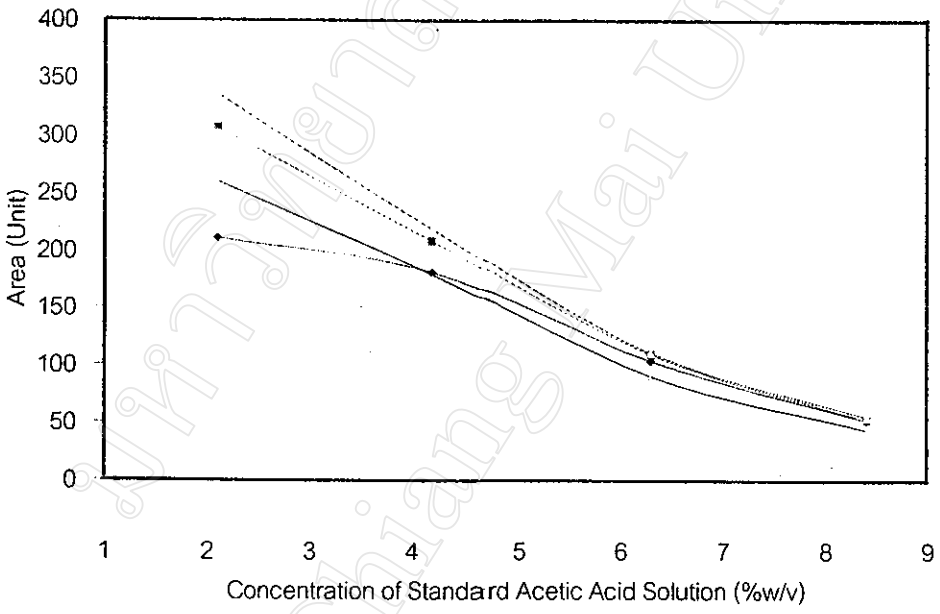

Figure 2.26 Effect of aspiration volume of phenolphthalein on peak: (×) 50, (◆) 60, (■) 70, (▲) 80 μL of 2 x 10^{-2} %(w/v) phenolphthalein.

Table 2.28 Effect of aspiration volume of phenolphthalein on linearity

Volume of phenolphthalein (μL)	Linear equation	Correlation coefficient
50	y = -35.170x + 327.3	0.9851
60	y = -26.363x + 274.6	0.9754
70	y = -41.092x + 384.2	0.9860
80	y = -45.265x + 417.0	0.9770

2.5.3.5 Effect of standard acetic acid aspiration volume

Using the conditions described in 2.5.3.4, a blank and a series of standard acetic acid solution were injected into the system but various aspiration volumes of acetic acid solution were changed in series of standard acetic acid solution. The results are shown in Tables 2.29, 2.30 and Figures 2.27, 2.28, so 50 μL of standard acetic acid solution was chosen to give a good linearity, high slope and this aspiration volume can be operated with a better accuracy than the over of 40 μL.

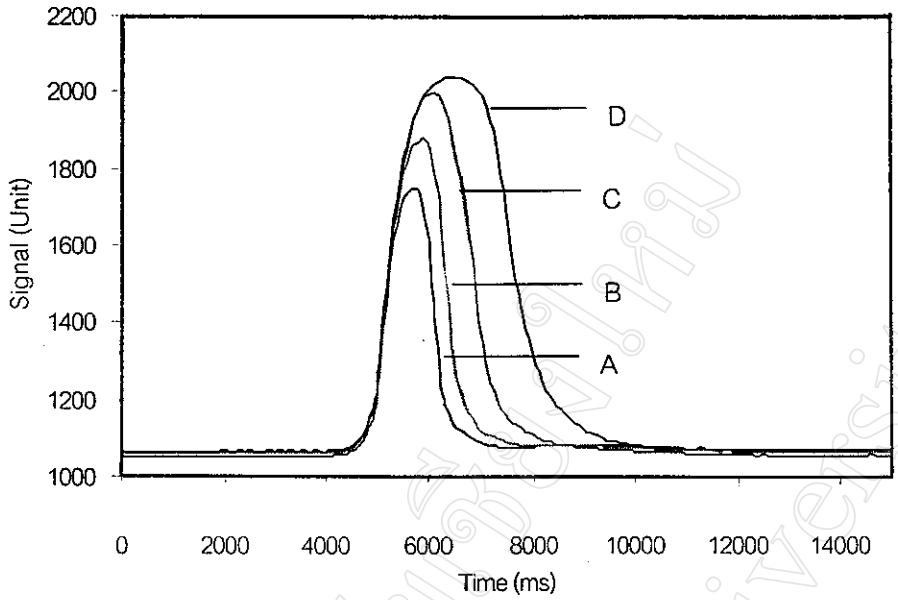

Figure 2.27 SIA peaks of different aspiration volumes of standard acetic acid (4%(w/v)): (A) 70, (B) 60, (C) 50 and (D) 40 μL of standard acetic acid solution.

Table 2.29 Effect of aspiration volume of standard acetic acid solution on peak area; mean of triplicate aspirations

Concentration of acetic acid (%w/v)	Area (Unit)			
	Volume of standard acetic acid solution (μL)			
	40	**50**	**60**	**70**
2.1	304.2	306.2	282.7	261.7
4.2	247.9	207.8	157.2	120.7
6.3	162.6	108.4	77.4	58.6
8.4	91.3	51.7	40.2	36.3

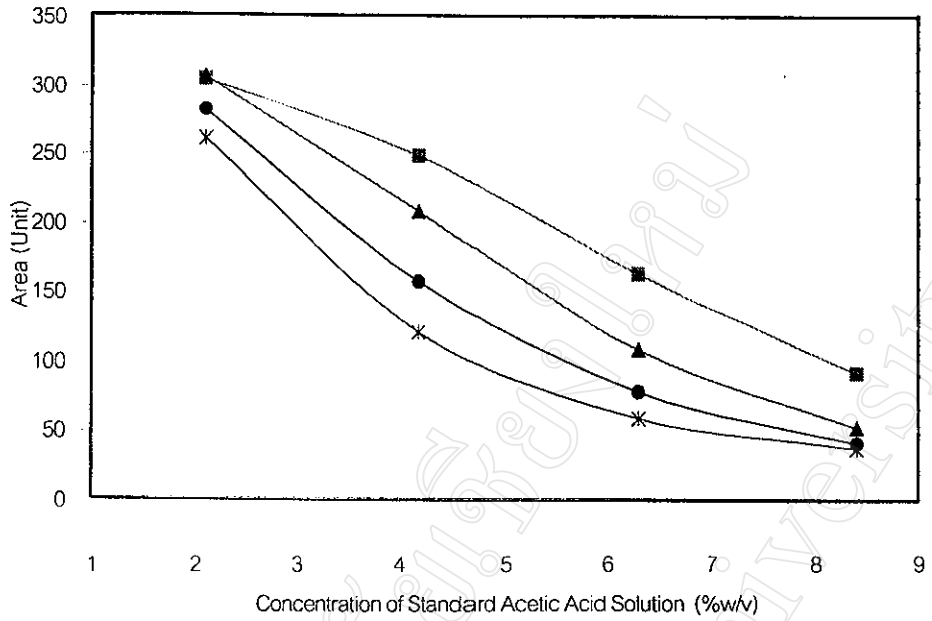

Figure 2.28 Effect of aspiration volume of standard acetic acid solution on peak area : (■) 40, (▲) 50, (●) 60 and (✳) 70 μL of standard acetic acid solution.

Table 2.30 Effect of aspiration volume of standard acetic acid solution on linearity

Volume of standard acetic acid solution (μL)	Linear equation	Correlation coefficient
40	y = -34.480x + 382.5	0.9943
50	y = -41.092x + 384.2	0.9860
60	y = -38.434x + 341.2	0.9435
70	y = -35.155x + 303.9	0.8833

2.5.3.6 Effect of pump speed (flow rate)

The effect of pump speed to drive the mixed solution through detector was studied. Using the conditions describe in 2.5.3.5, a blank and a series of standard acetic acid solution were injected to the system. In each series of standard acetic acid, pump speed was

changed. The results are shown in Tables 2.31, 2.32 and Figures 2.29, 2.30. The results indicated that the peak area increases when pump speed decreases.

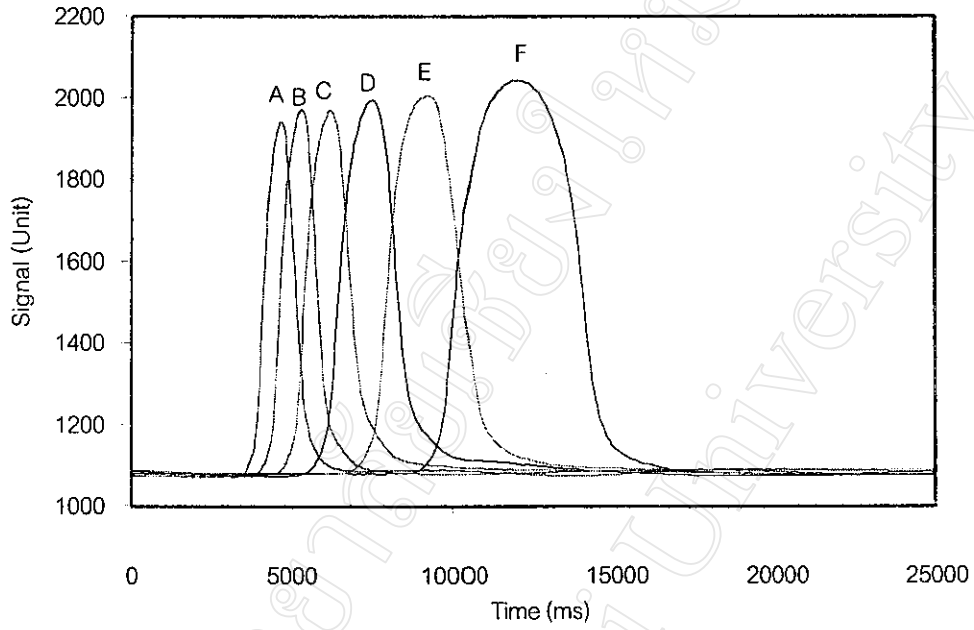

Figure 2.29 SIA peaks of different pump speeds (a standard acetic acid concentration of 4% (w/v)): (A) 200, (B) 175, (C) 150, (D) 125, (E) 100 and (F) 75 μL/s of pump speed.

Table 2.31 Effect of pump speed on peak area; mean of triplicate aspirations

Concentration of acetic acid (%w/v)	Area (Unit)					
	Pump speed (μL/s)					
	75	**100**	**125**	**150**	**175**	**200**
2	322.2	257.9	211.7	179.2	169.1	312.1
4	281.8	212.6	190.9	160.4	136.4	267.3
6	187.0	147.1	131.0	99.5	92.3	183.1
8	121.4	87.6	70.6	64.2	56.0	115.6

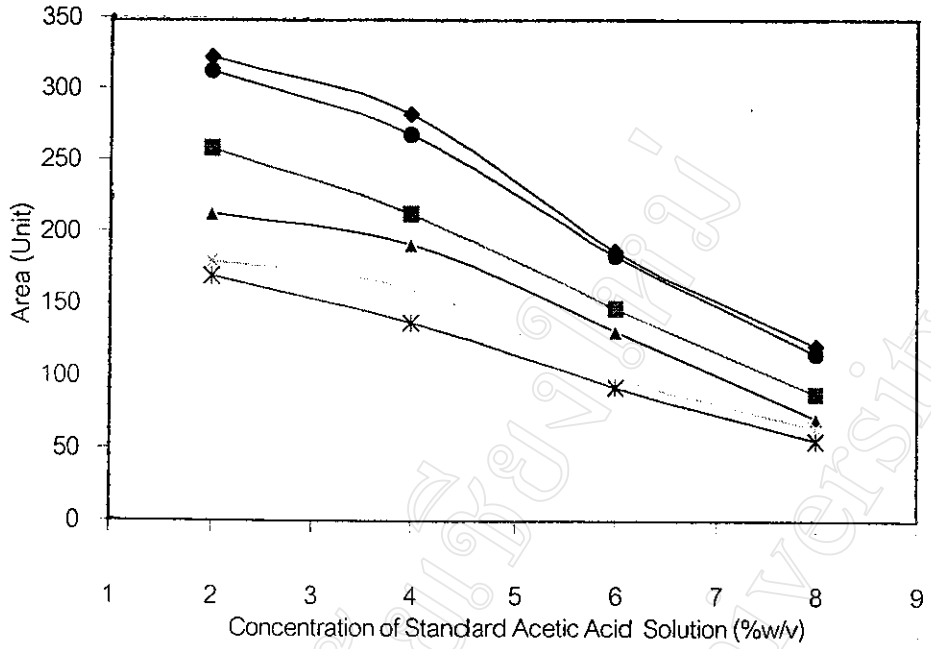

Figure 2.30 Effect of pump speed on peak area : (◆) 75, (■) 100, (▲) 125, (×) 150, (✳) 175 and (●) 200 μL/s of pump speed.

Table 2.32 Effect of pump speed on linearity

Pump speed (μL/s)	Linear equation	Correlation coefficient
75	y = -34.856x + 402.4	0.9795
100	y = -33.678x + 387.9	0.9875
125	y = -28.824x + 320.5	0.9949
150	y = -24.152x + 271.8	0.9615
175	y = -20.288x + 227.2	0.9652
200	y = -19.162x + 209.3	0.9970

2.5.3.7 Summary of conditions used

The SIA system used is depicted in Figures 2.18 and the optimum conditions are summarized in Table 2.33.

Table 2.33 The optimized SI condition for acetic acid determination

Parameter	Conditions
Aspiration volume of 0.8 M sodium hydroxide solution	70 μL
Aspiration volume of standard acetic acid solution	50 μL
Aspiration volume of 2 x 10^{-2} % (w/v) phenolphthalein solution	70 μL
Speed of pump	125 μL/s

2.5.3.8 Precision

Using the optimum SIA conditions described in 2.5.3.7, the precision of the suitable conditions were studied by repeating aspiration of 5% (w/v) and 4% (w/v) of standard acetic acid solution for 11 replicates. The results are shown in Table 2.34 and Figures 2.31, 2.32. The percentages of relative standard deviations (%RSD) are 4.8% and 6.3 % respectively.

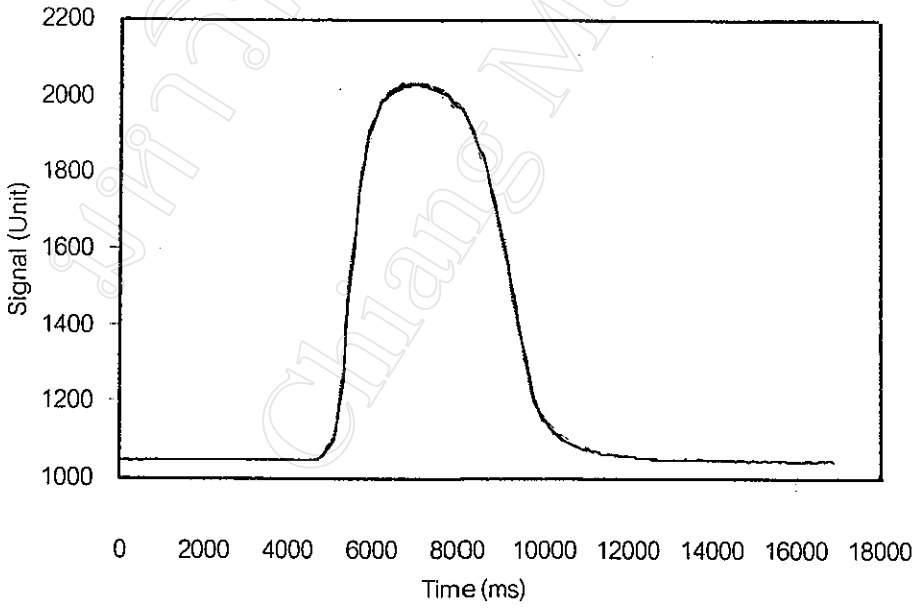

Figure 2.31 SIA peaks for precision study using 5%(w/v) acetic acid (n=11).

Table 2.34 Precision study using 5%(w/v) acetic acid and 4%(w/v) acetic acid (n=11)

Number of injection	Area (Unit)	
	5%(w/v) acetic acid	4%(w/v) acetic acid
1	232.9	224.7
2	236.5	250.1
3	243.8	221.3
4	247.0	254.6
5	249.1	261.4
6	233.9	262.3
7	248.6	233.3
8	248.6	236.1
9	235.6	229.3
10	227.1	232.9
11	211.9	225.4
Average	237.7	239.2
Standard deviation	11.4	15.1
RSD	0.05	0.06
% RSD	4.8	6.3

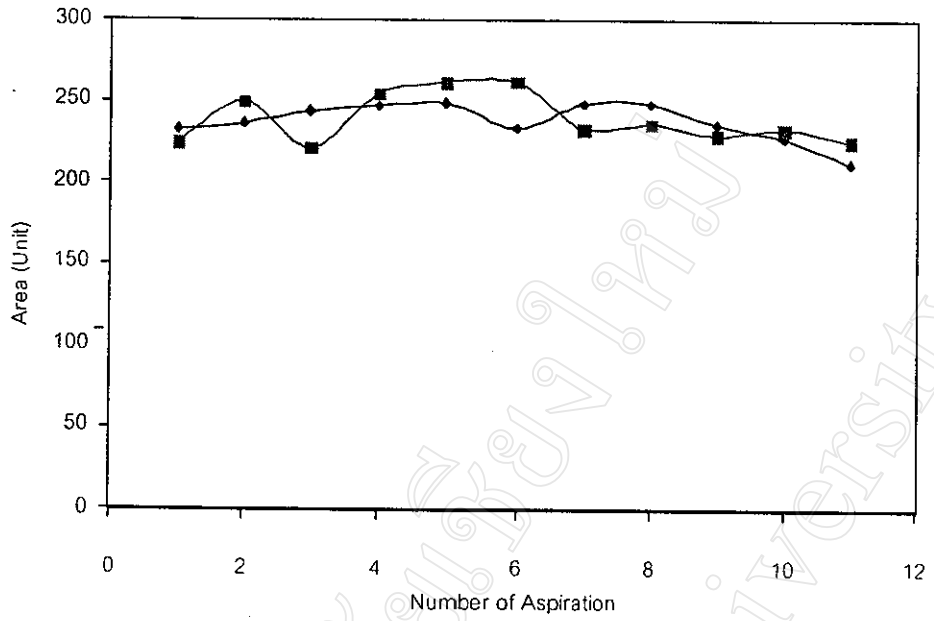

Figure 2.32 Precision study (n=11): (◆) 5%(w/v) acetic acid and (■) 4%(w/v) of standard acetic acid.

2.5.3.9 Determination of acetic acid in vinegar samples

The optimized system was applied to the determination of acetic acid in locally commercial vinegar samples. A standard titration method [APPENDIX B] was also carried out. The results are shown in Tables 2.35, 2.36 and Figures 2.33, 2.34, and 2.35. The comparison was evaluated by t-test [24]. The calculated t-test value was 1.16. The critical value of t-test was 2.20 (where t has 11 degrees of freedoms) at confidence interval of 95% and since the calculated value of t-test was less than the critical value. These results from the two recommended methods are not different significantly (confidence interval of 95%).

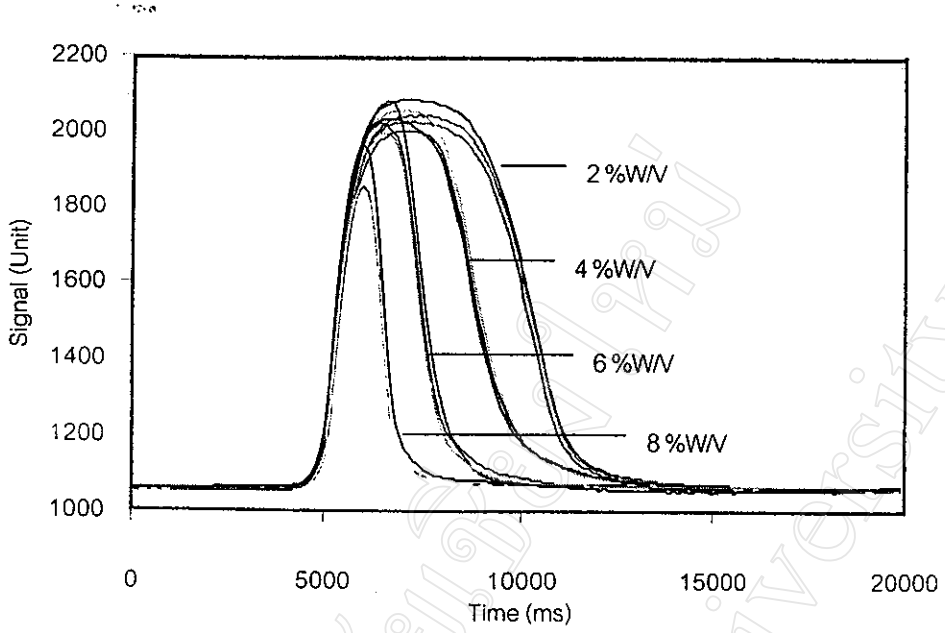

Figure 2.33 SIA peaks for calibration curve of acetic acid.

Table 2.35 Calibration graph of acetic acid

Concentration of acetic acid (%w/v)	Area (Unit)			
	1	2	3	Average
2	303.9	319.2	320.3	314.5
4	254.7	235.0	241.0	243.6
6	174.6	162.7	144.3	160.5
8	91.8	92.4	88.1	90.8

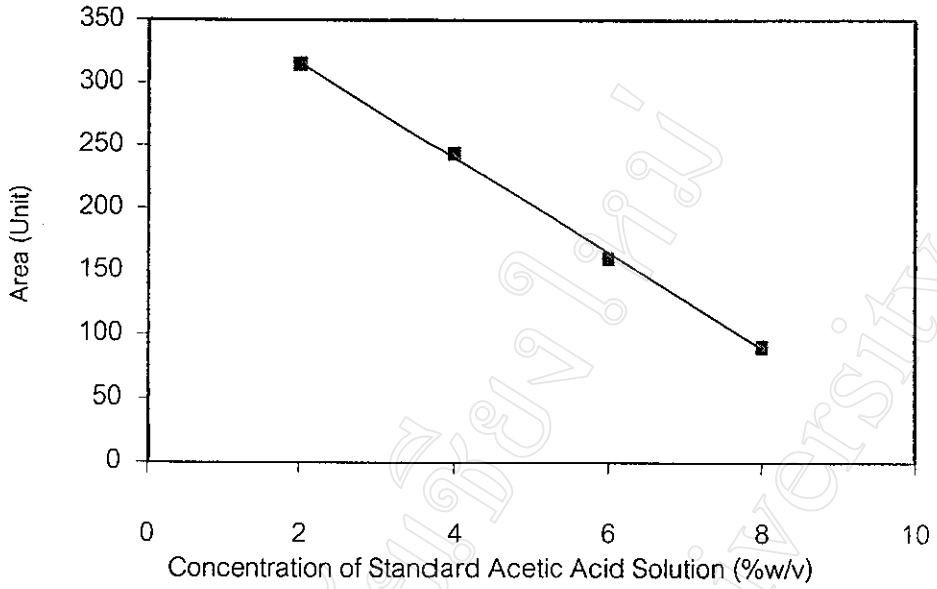

Figure 2.34 Calibration curve of acetic acid: y = -37.709x + 390.9, R^2= 0.9989.

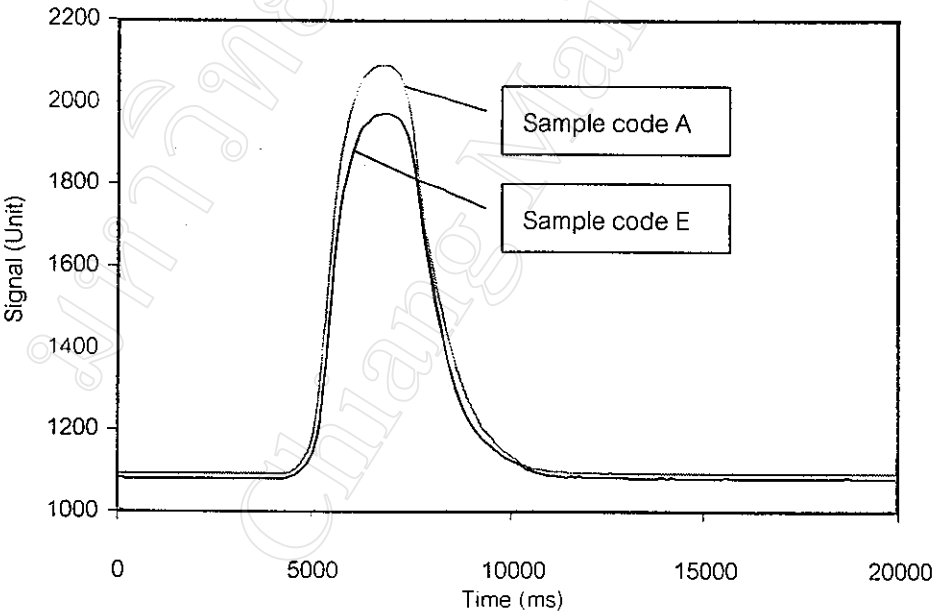

Figure 2.35 SIA peaks for vinegar samples (codes A and E).

Table 2.36 Determination of acetic acid in locally commercial vinegar samples

Sample code	Concentration of acetic acid (%w/v) by using different methods	
	SIA method	Titrimetric method
A	5.1	5.2
B	5.5	5.1
C	5.4	5.1
D	5.5	5.1
E	5.5	5.1
F	6.0	5.3
G	5.7	5.2
H	6.1	5.8
I	5.1	5.1
J	4.4	4.9
K	4.5	4.8
L	5.1	5.0