APPENDICES

APPENDIX A

Reference Tartaric acid

The reference tartaric acid for interference study of stopped-flow system in this research is listed in Table A.1.

Table A.1 Acids naturally present in fruits (* = % of the total acid in the fruit) [45]

Fruits	Predominant acid	Secondary acids
Apple	Malic acid (95%*)	Tartaric acid, Fumaric acid
Apricot	Malic acid (70%*)	Citric acid, Tartaric acid
Cherry	Malic acid (94%*)	Tartaric acid
Grape	Malic acid (60%*)	Tartaric acid
Grapefruit	Citric acid	Malic acid
Guava	Citric acid	Malic acid
Lime, Lemon	Citric acid	Malic acid
Mongo	Citric acid	Malic acid, Tartaric acid
Orange	Citric acid	Malic acid
Peach	Malic acid (73%*)	Citric acid
Pear	Malic acid (77%*)	Citric acid
Pineapple	Citric acid	Malic acid
Raspberry	Citric acid	Malic acid, Tartaric acid
Strawberry	Citric acid	Malic acid, Tartaric acid
Tamarind	Tartaric acid	Citric acid, Malic acid
Watermelon	Malic acid (99%*)	Fumaric acid

APPENDIX B

Determination of vitamin C in fruit samples

1. Standardize the 2,6-dichlorophenol indophenol solution

Vitamin C in titration method was prepared at 1000 mg/L by dissolving 100 mg of ascorbic acid reference standard, with the extracting solution (metaphosphoric acid - acetic acid solution) and the final volume was adjusted to 100 mL. Immediately transfer 2 mL of ascorbic acid solution to Erlenmeyer flask containing 5 mL of extracting solution and titrate rapidly with the dichlorophenol indophenol solution until a distinct rose-pink color persists for at least 5 sec.

The solution of blank titration was prepare by mixing 7 mL of the extracting solution with dichlorophenol indophenol solution at the volume equal to that used in the titration of the ascorbic acid solution in the previous step. The concentration of the standard solution is expressed in terms of its equivalent in milligrams of ascorbic acid.

2. Assay procedure

The sample was dissolved in a sufficient volume of extracting solution so that each milliliter of solution contains approximately 1 mg of ascorbic acid. A 2 mL portion was transferred to an Erlenmeyer flask. A 5 mL portion of extracting solution was added and the mixture was titrated with standard dichlorophenol indophenol solution until a rose-pink color was obtained.

An example of the calculation of the amount of vitamin C in some fruit samples found by titrimetric method is shown below.

Guava fruit sample

A 2 mL portion of stock ascorbic acid solution (1000 mg/L) was transferred to Erlenmeyer flask. It contains approximately 2 mg of ascorbic acid.

Titrate with dichlorophenol	16.38	mL	=	ascorbic acid	2	mg
Titrate with dichlorophenol	1.00	mL	=	ascorbic acid	(2/16.38)	mg
			=	0.1221 mg		
Titrate with dichlorophenol	1.00	mL	=	ascorbic acid	0.1221	mg
Titrate with dichlorophenol	16.83	mL	=	fruit sample	(0.1221×16.83)	mg
			=	2.0549 mg		
Therefore, 2 mL of sample solution			=	fruit sample	(2.0549/2) ×1000	mg
			=	1027.45 mg/L		

A guava fruit sample weight	=	495.54 g		
The solution of fruit sample weight	=	300.31 g		
The volume of 25 mL weight	=	19.33 g		
The volume of 25 mL + solution sample	=	25.84 g		
• Calculation of density	=	weigh / volume		
	=	25.84 / 25	=	1.0338
Therefore, total sample solution	=	300.31 / 1.0338	=	290.50 ml

From the volume of dichlorophenol solution used in titration with the fruit sample solution, the concentration of the standard solution is expressed in terms of its equivalent in 1027.45 mg/L of ascorbic acid.

Fruit sample solution	1000	mL	=	ascorbic acid 1027.45 mg
Fruit sample solution	290.50	mL	=	ascorbic acid (290.50×1027.45)/ 1000
			=	298.47 mg

A guava fruit sample weight = 495.54 g

Calculation of the concentration of the ascorbic acid in guava fruit sample

= (298.47 mg / 0.4955 kg)

= 602.32 mg / kg

The another values of some fruit samples in this the calculation are listed in Table B.1

Table B.1 The detail of fruit samples

Sample number	Fruit samples	Weight of fruit samples (g)	Volume of fruit samples (ml)	Density (g/mL)
1	Mango 1	338.23	158.43	1.0400
2	Mango 2	383.38	196.51	1.0536
3	Apple red 1	235.02	131.77	1.0496
4	Apple red 2	175.51	125.24	1.0548
5	Apple fuji 1	219.16	162.39	1.0432
6	Apple fuji 2	230.04	161.02	1.0424
7	Apple green	214.97	130.72	1.0528
8	Chinese pear 1	226.28	166.97	1.0364
9	Chinese pear 2	211.72	152.83	1.0360
10	Rose-apple red 1	266.53	176.27	1.0324
11	Rose-apple red 2	244.38	172.59	1.0348
12	Rose-apple green	196.84	122.19	1.0292
13	Pineapple 1	1166.41	490.87	1.0716
14	Pineapple 2	1197.67	468.29	1.0392
15	Tangerine 1	382.47	188.46	1.0510
16	Tangerine 2	442.92	174.39	1.0524
17	Star fruit 1	272.72	187.05	1.0264
18	Star fruit 2	272.72	187.05	1.0264
19	Lemon 1	200.47	188.49	1.0190
20	Lemon 2	435.87	110.60	1.0380
21	Strawberry 1	96.93	66.18	1.0268
22	Strawberry 2	138.66	96.85	1.0300
23	Jujube 1	355.32	232.88	1.0528
24	Jujube 2	340.85	225.46	1.0490
25	Guava 1	489.92	321.48	1.0320
26	Guava 2	495.54	300.31	1.0338

APPENDIX C

Stopped-FIA gram

The stopped-FIA gram from the system was used as the parameter study for determination of vitamin C in some fruit samples.

1. Effect of sodium molybdate concentration

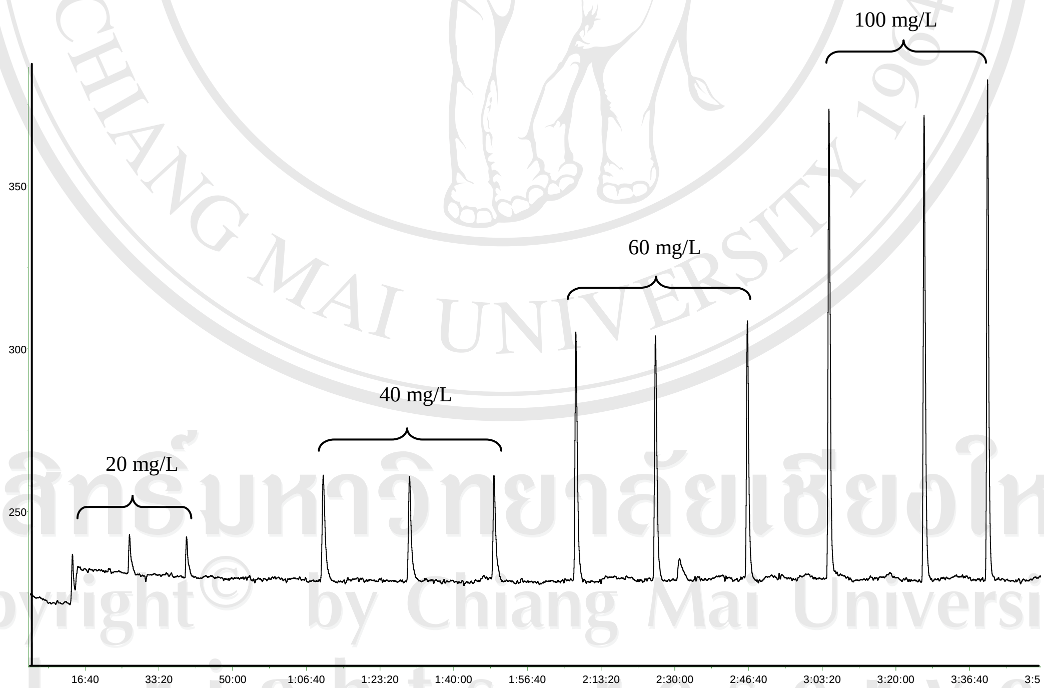

Figure 1 Stopped-FIA gram of $Na_2MoO_4.2H_2O$ concentration at 0.34×10^{-1} M

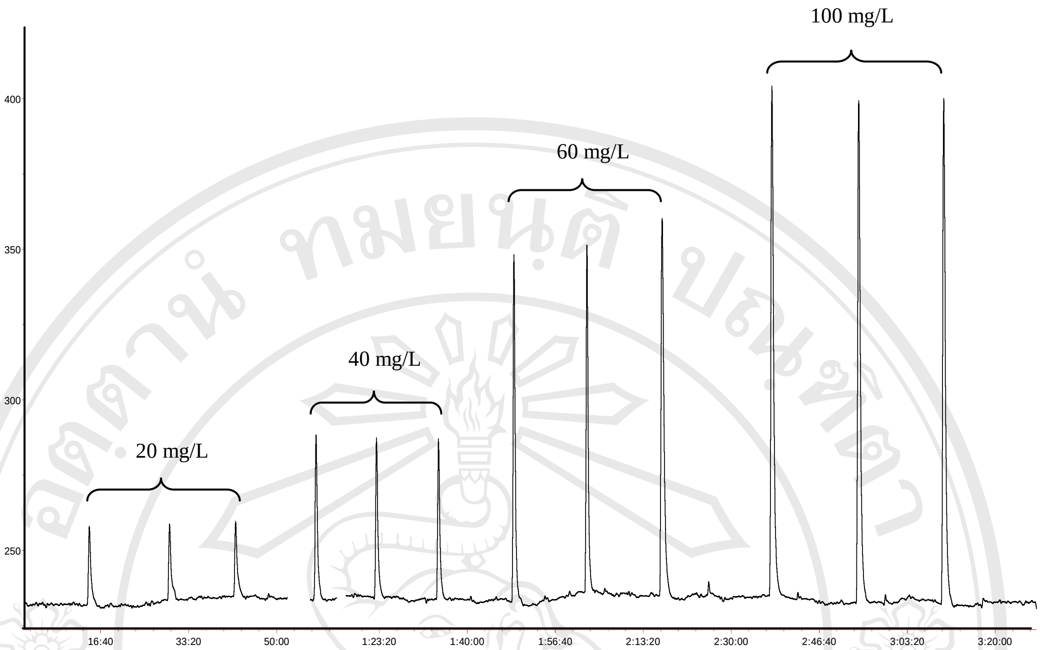

Figure 2 Stopped-FIA gram of $Na_2MoO_4.2H_2O$ concentration at 0.68×10^{-1} M

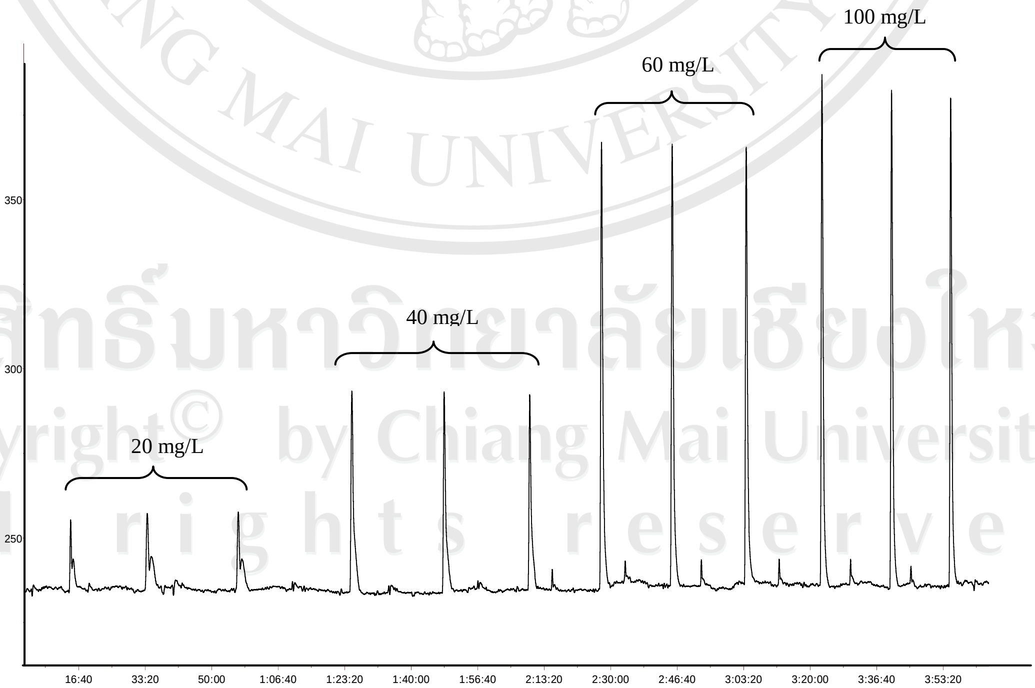

Figure 3 Stopped-FIA gram of $Na_2MoO_4.2H_2O$ concentration at 1.36×10^{-1} M

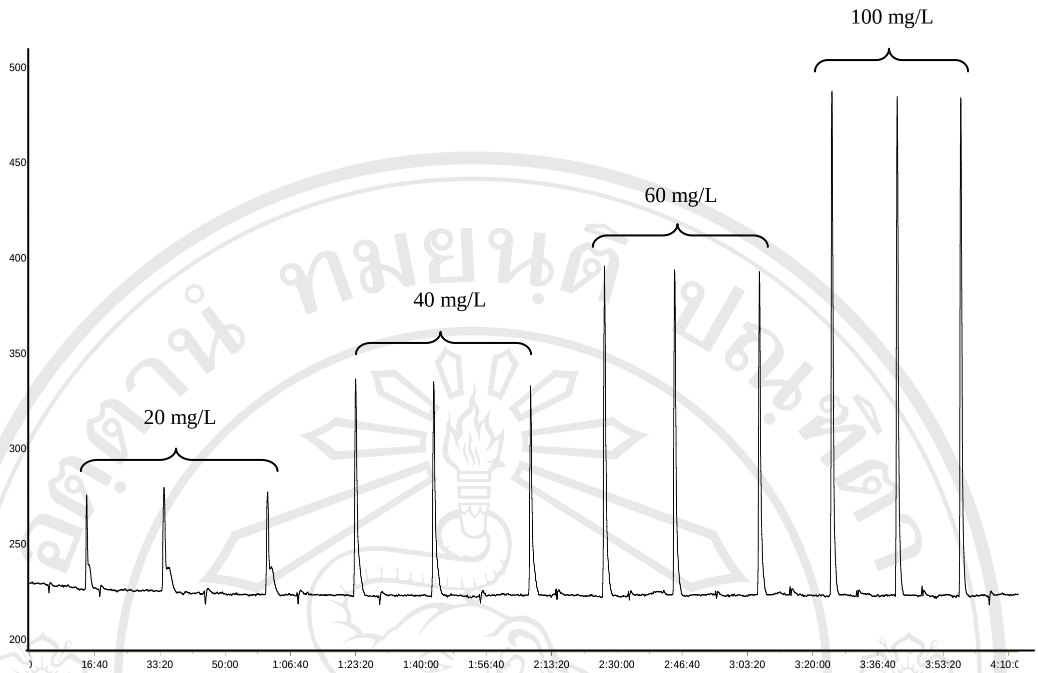

Figure 4 Stopped-FIA gram of $Na_2MoO_4.2H_2O$ concentration at 2.04×10^{-1} M

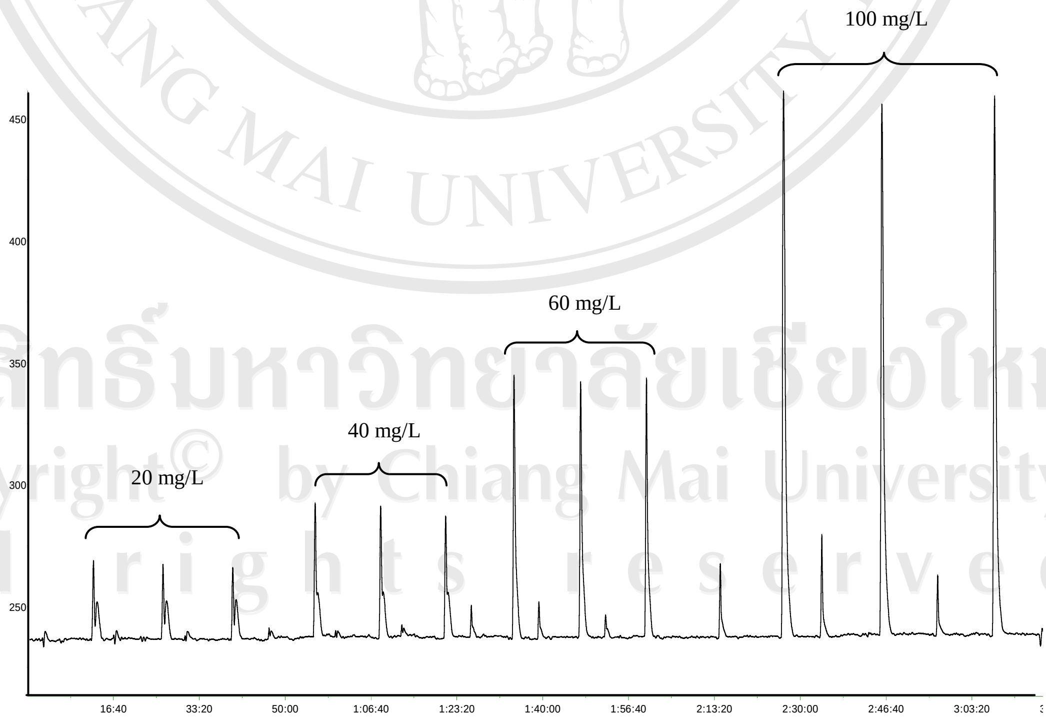

Figure 5 Stopped-FIA gram of $Na_2MoO_4.2H_2O$ concentration at 3.40×10^{-1} M

2. Effect of potassium dihydrogen phosphate concentrations

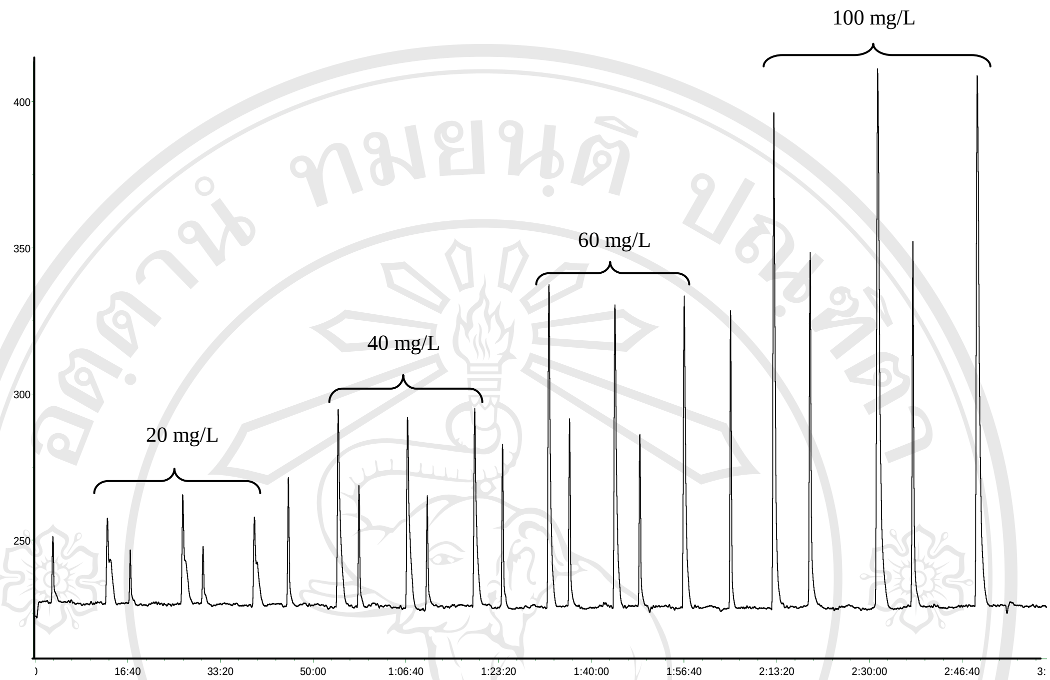

Figure 6 Stopped-FIA gram of KH_2PO_4 concentration at 0.07×10^{-1} M

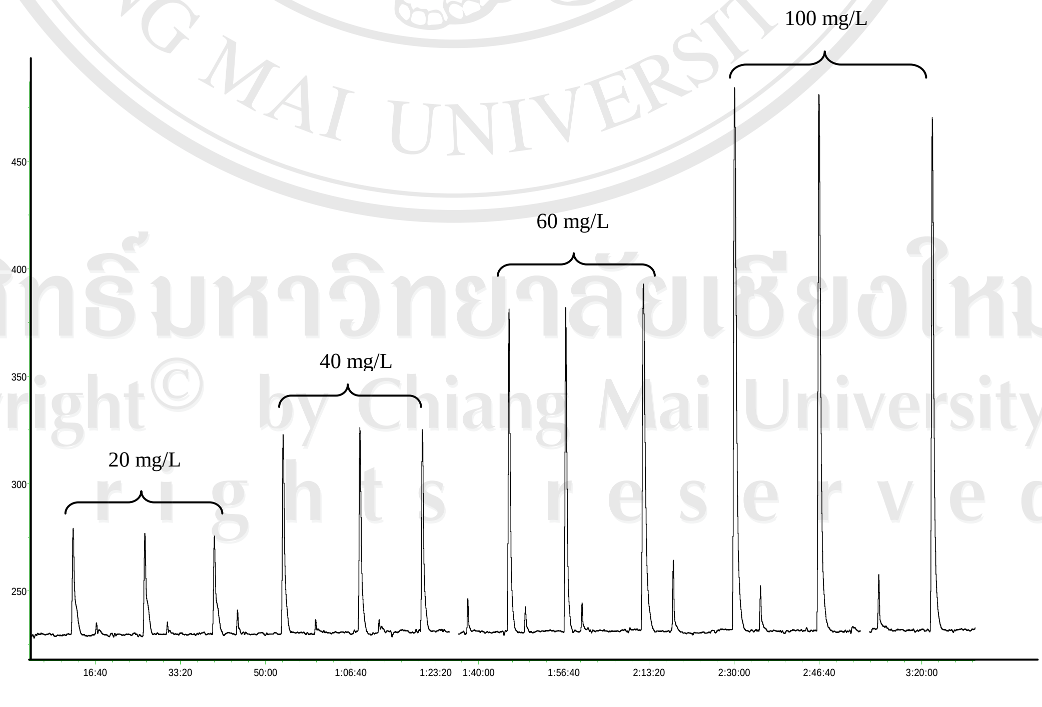

Figure 7 Stopped-FIA gram of KH_2PO_4 concentration at 0.14×10^{-1} M

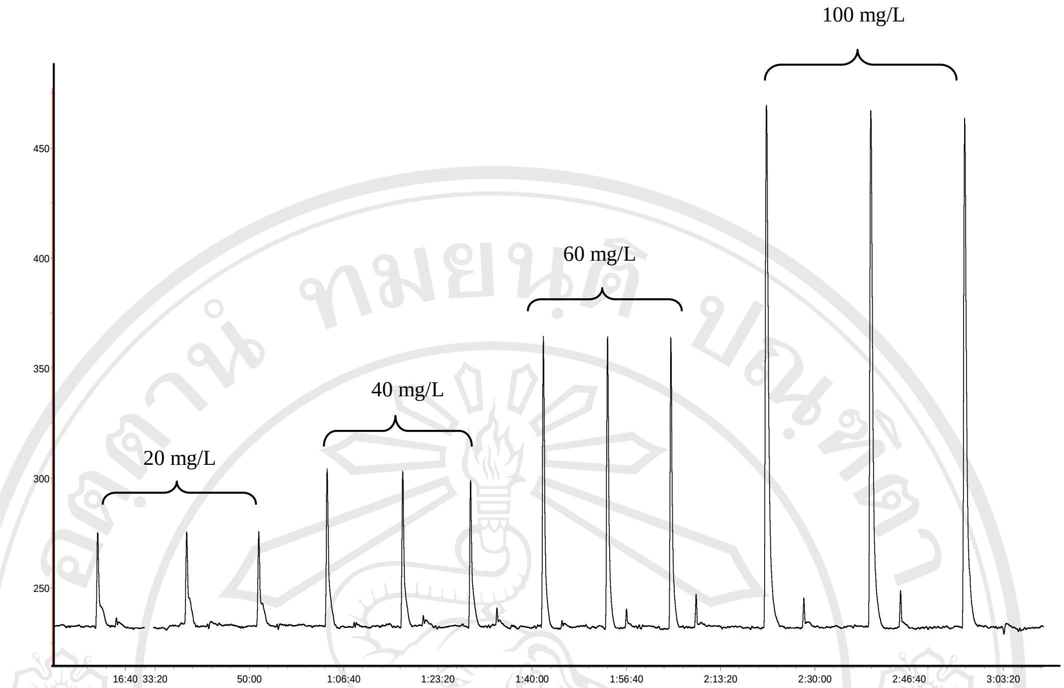

Figure 8 Stopped-FIA gram of KH_2PO_4 concentration at 0.28×10^{-1} M

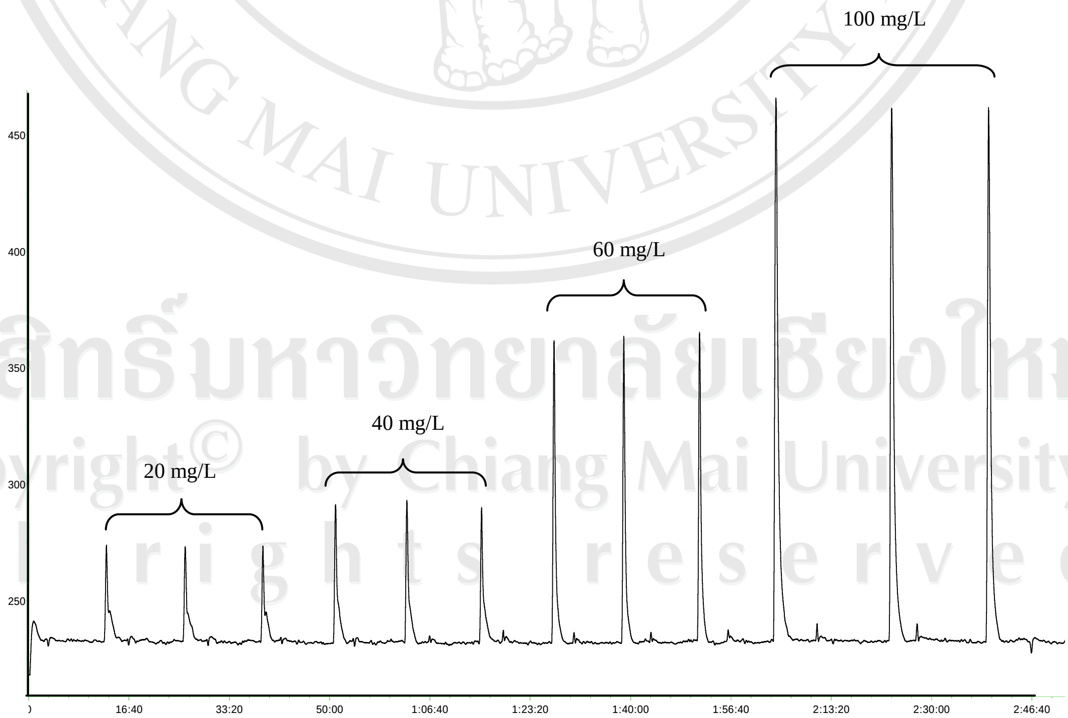

Figure 9 Stopped-FIA gram of KH_2PO_4 concentration at 0.42×10^{-1} M

3. Effect of sulfuric acid concentration

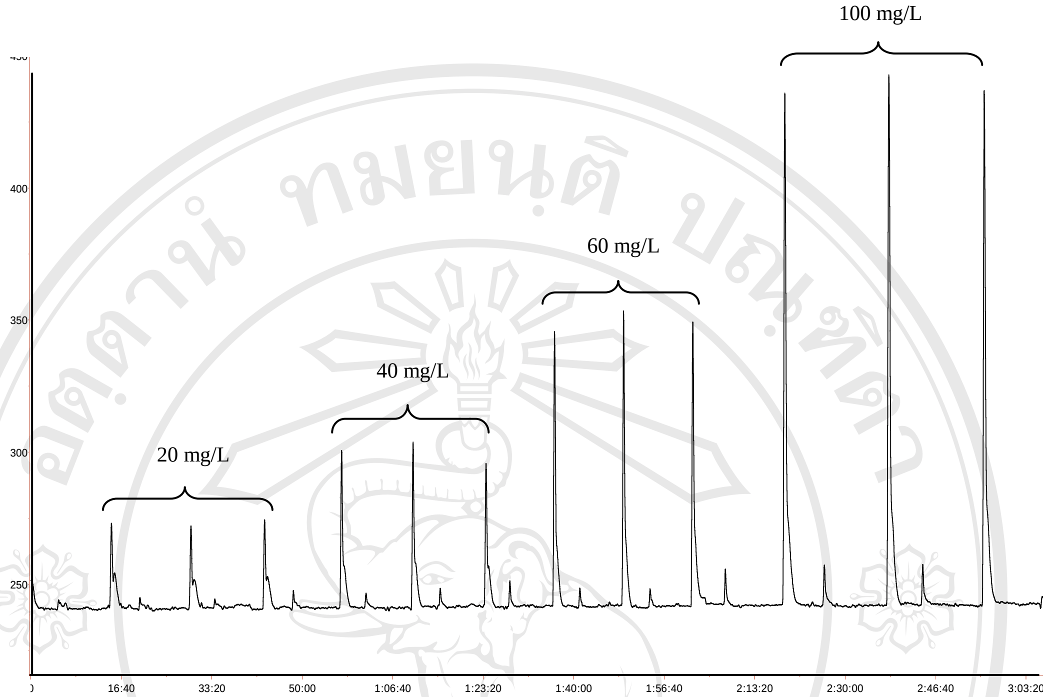

Figure 10 Stopped-FIA gram of H_2SO_4 concentration at $0.37{\times}10^{-1}$ M

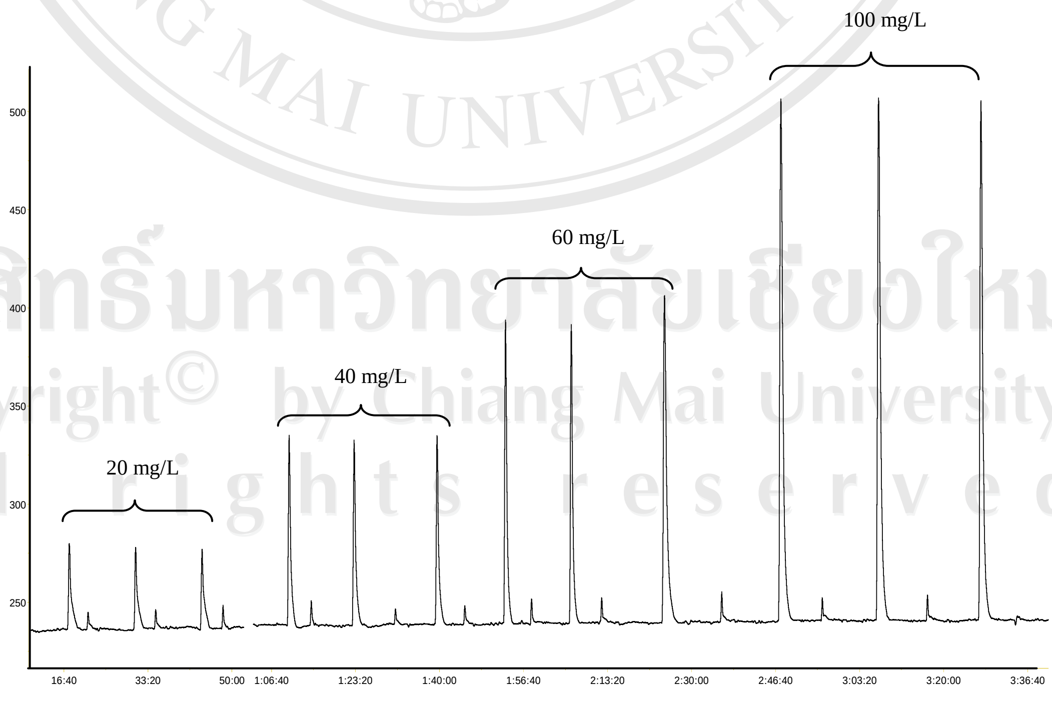

Figure 11 Stopped-FIA gram of H_2SO_4 concentration at $0.55{\times}10^{-1}$ M

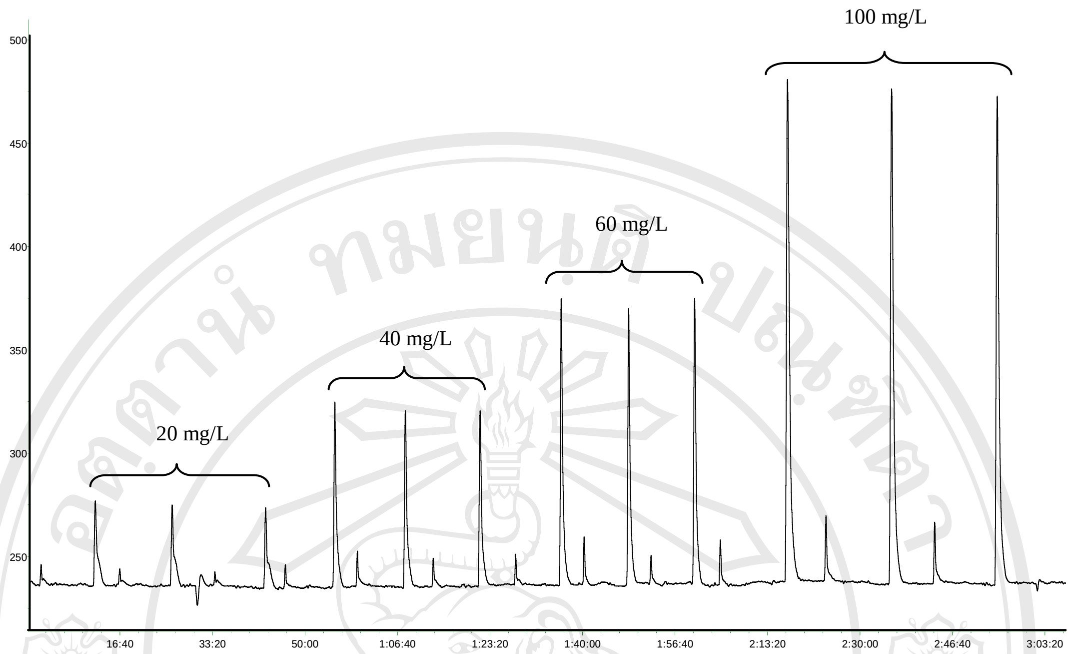

Figure 12 Stopped-FIA gram of H_2SO_4 concentration at 0.74×10^{-1} M

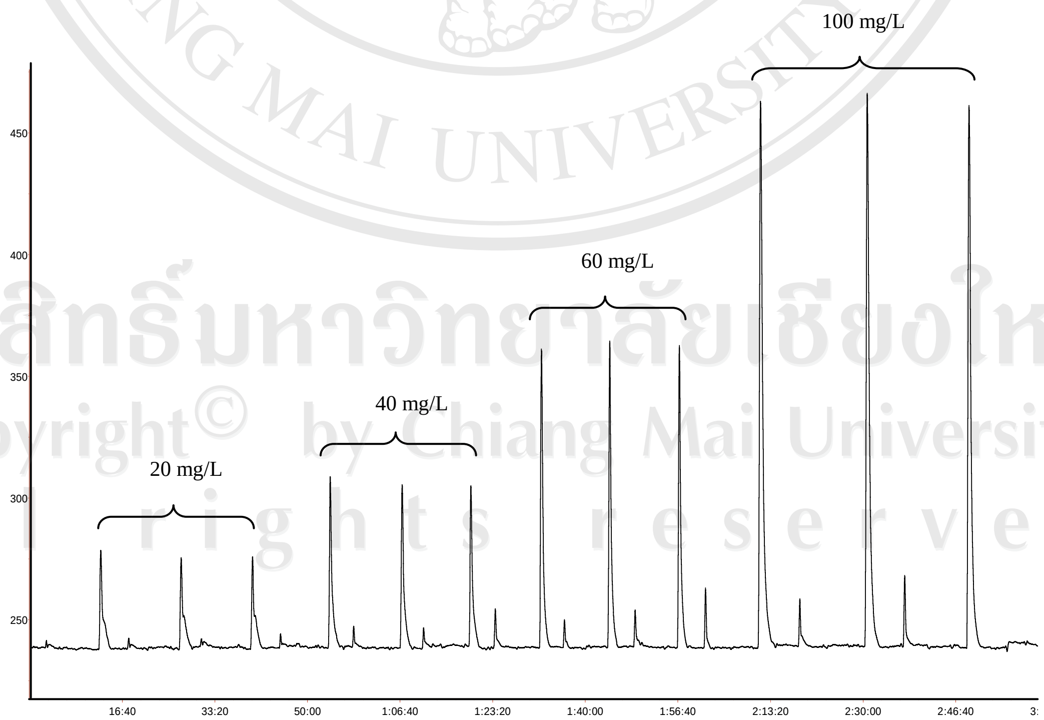

Figure 13 Stopped-FIA gram of H_2SO_4 concentration at 0.92×10^{-1} M

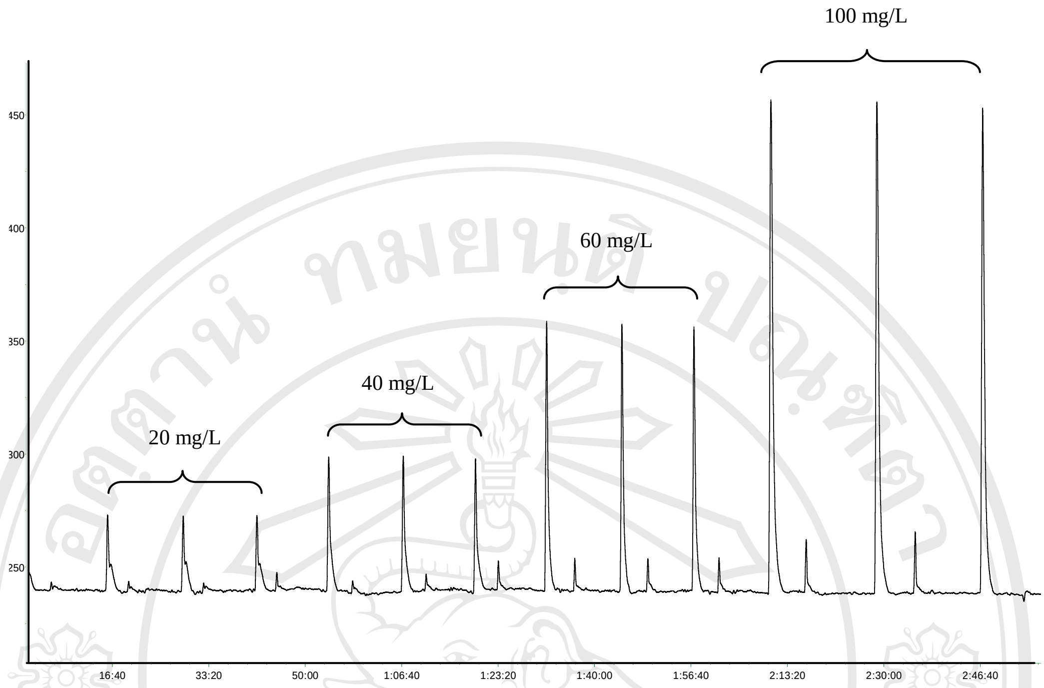

Figure 14 Stopped-FIA gram of H_2SO_4 concentration at 1.10×10^{-1} M

4. Effect of stopping time in stopping coil (MC2)

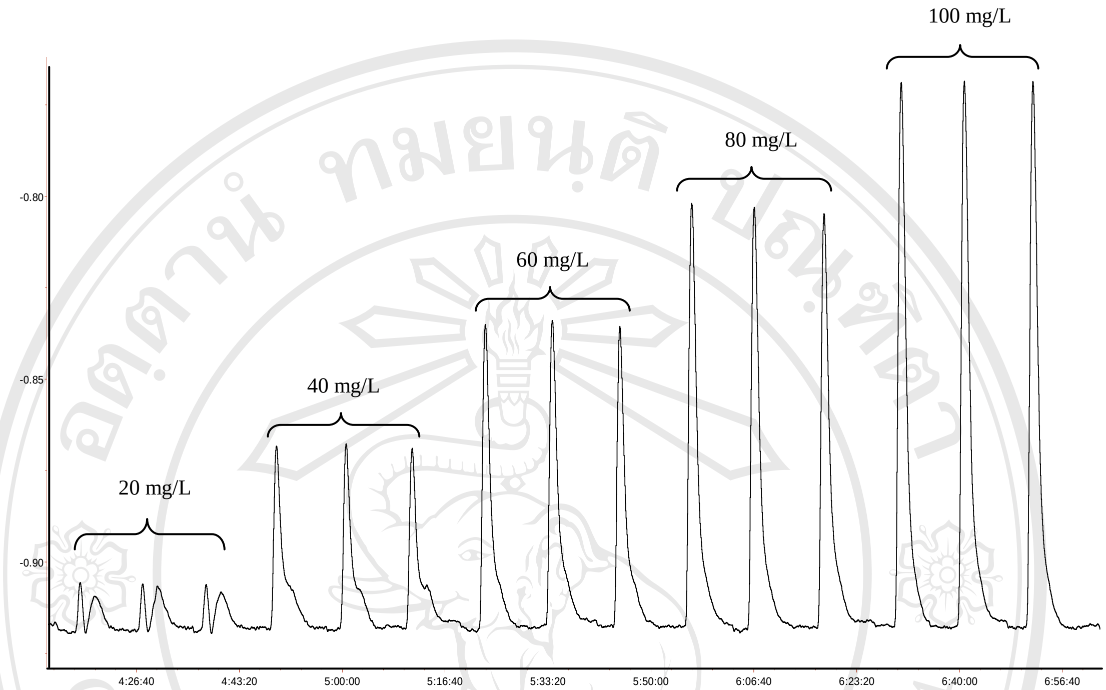

Figure 15 Stopped-FIA gram non-stop time

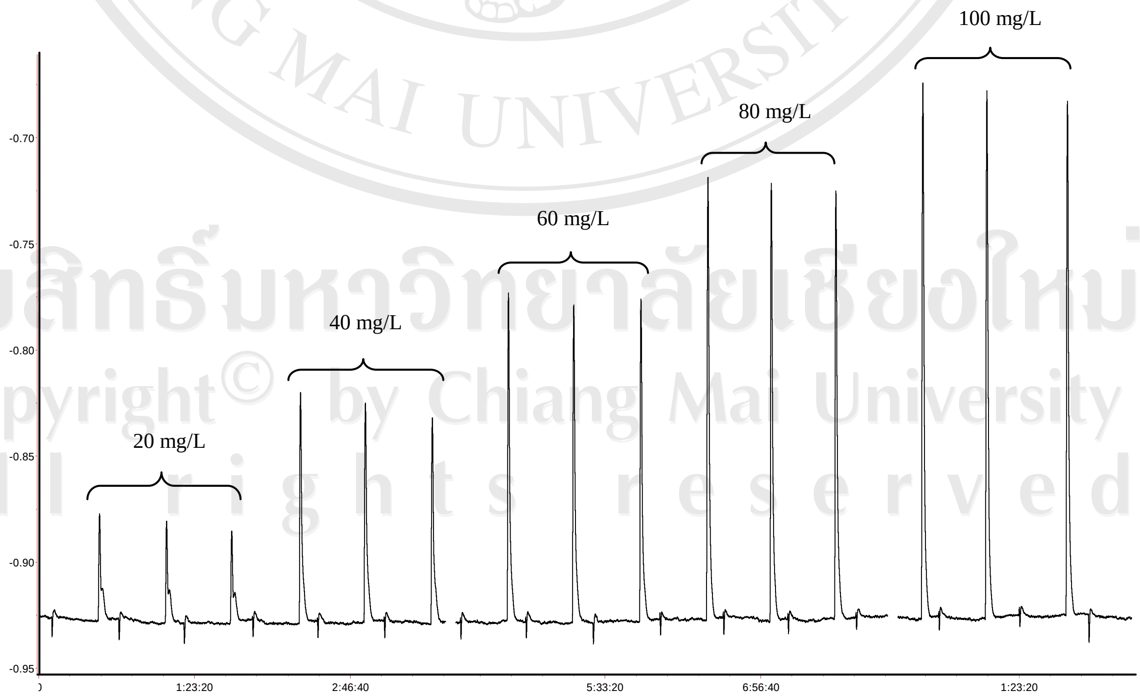

Figure 16 Stopped-FIA gram for 4 minute stopping time

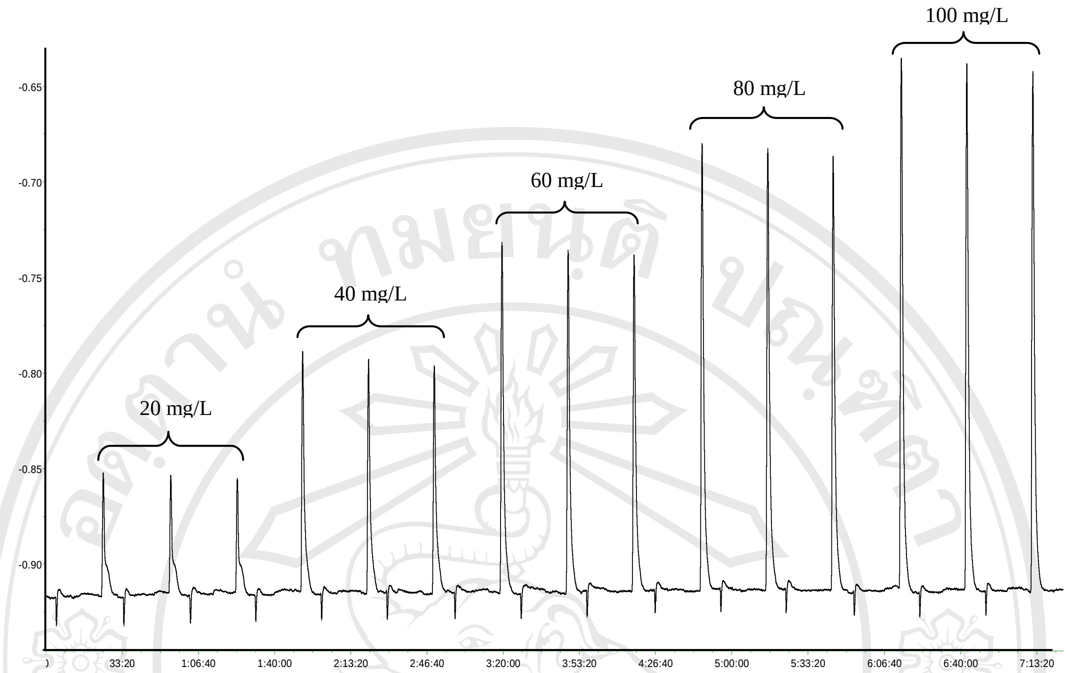

Figure 17 Stopped-FIA gram for 5 minute stopping time

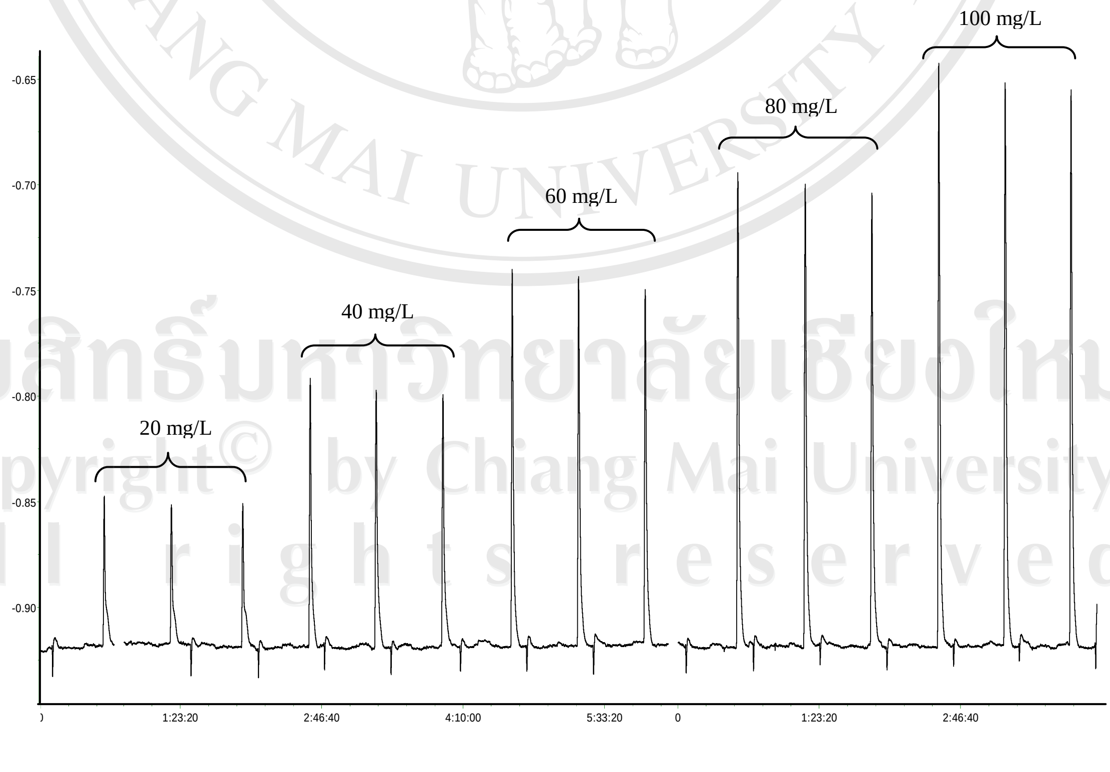

Figure 18 Stopped-FIA gram for 6 minute stopping time

5. The application of vitamin C in some fruit samples

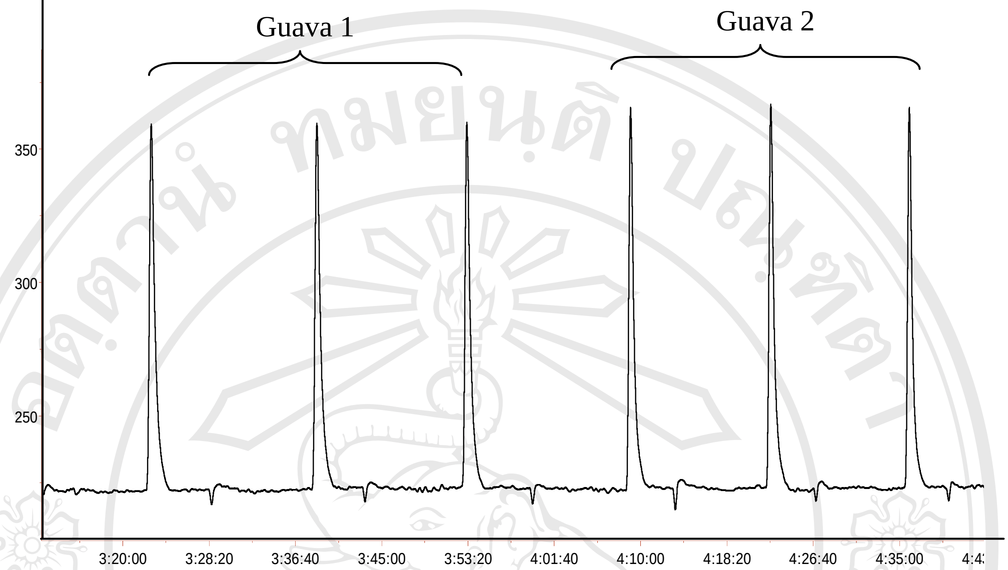

Figure 19 Stopped-FIA gram of guava samples

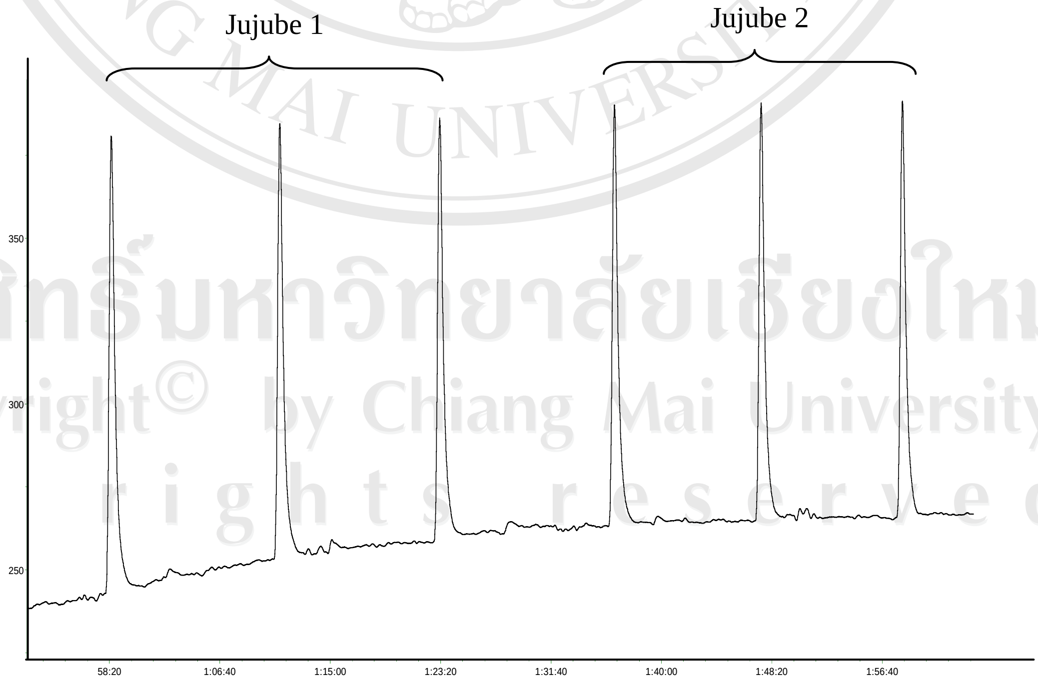

Figure 20 Stopped-FIA gram of jujube samples

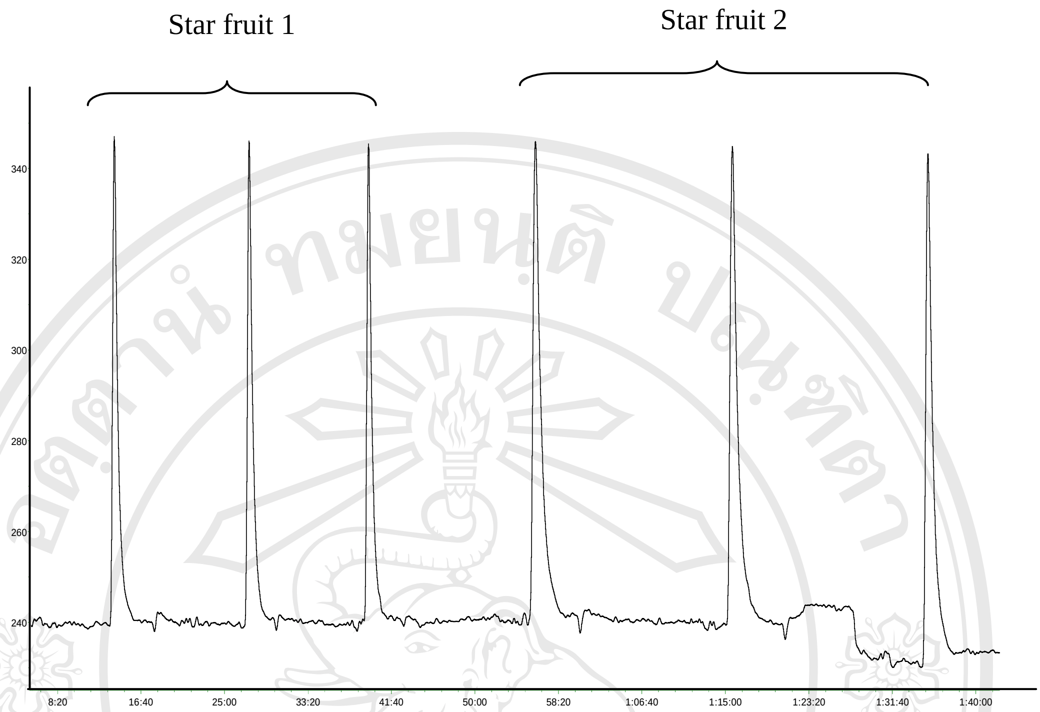

Figure 21 Stopped-FIA gram of star fruit samples

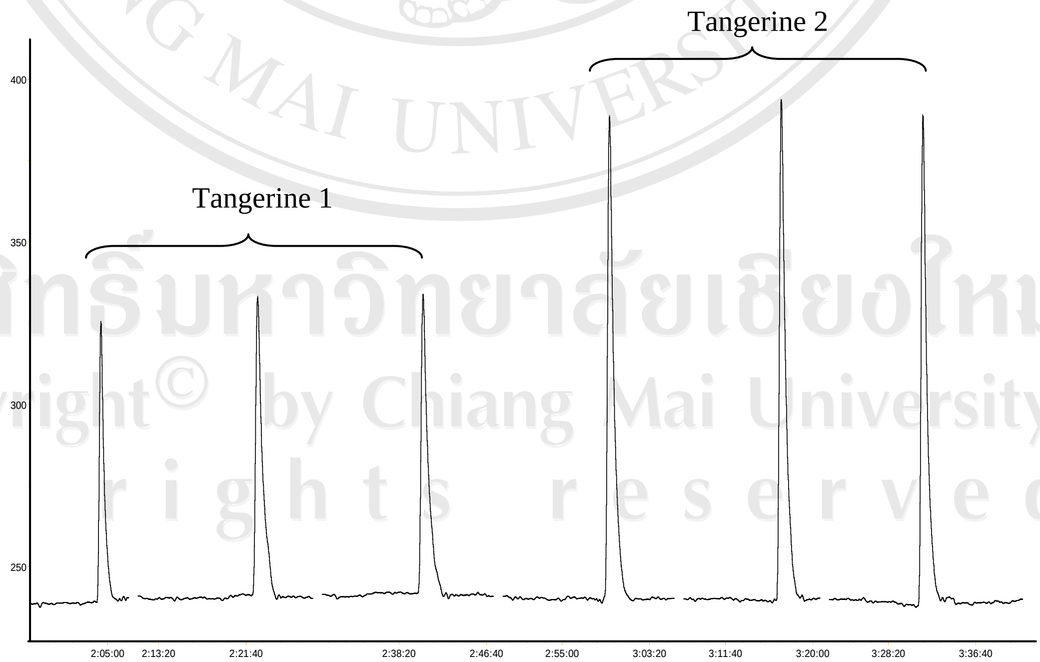

Figure 22 Stopped-FIA gram of tangerine samples

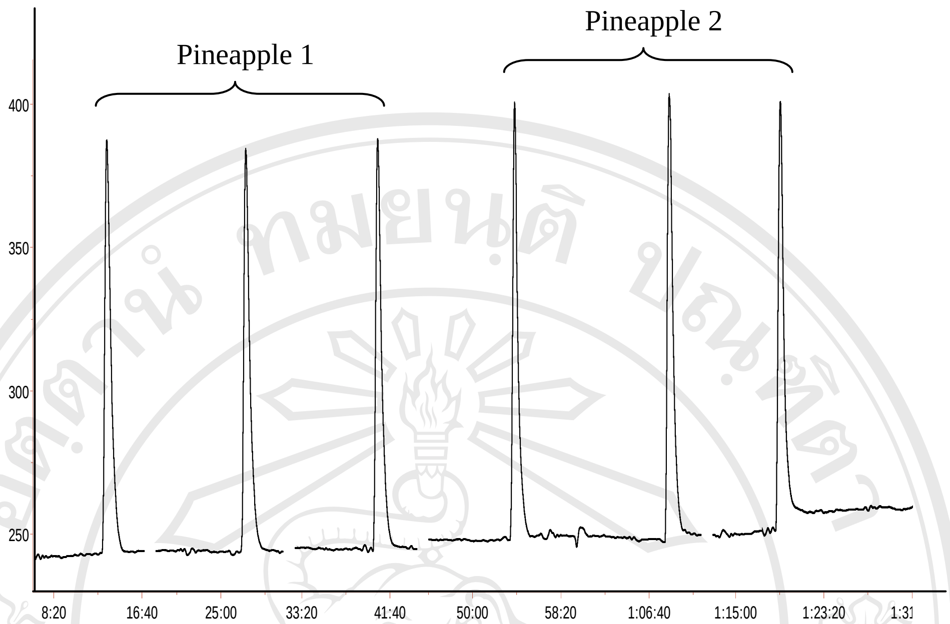

Figure 23 Stopped-FIA gram of pineapple samples

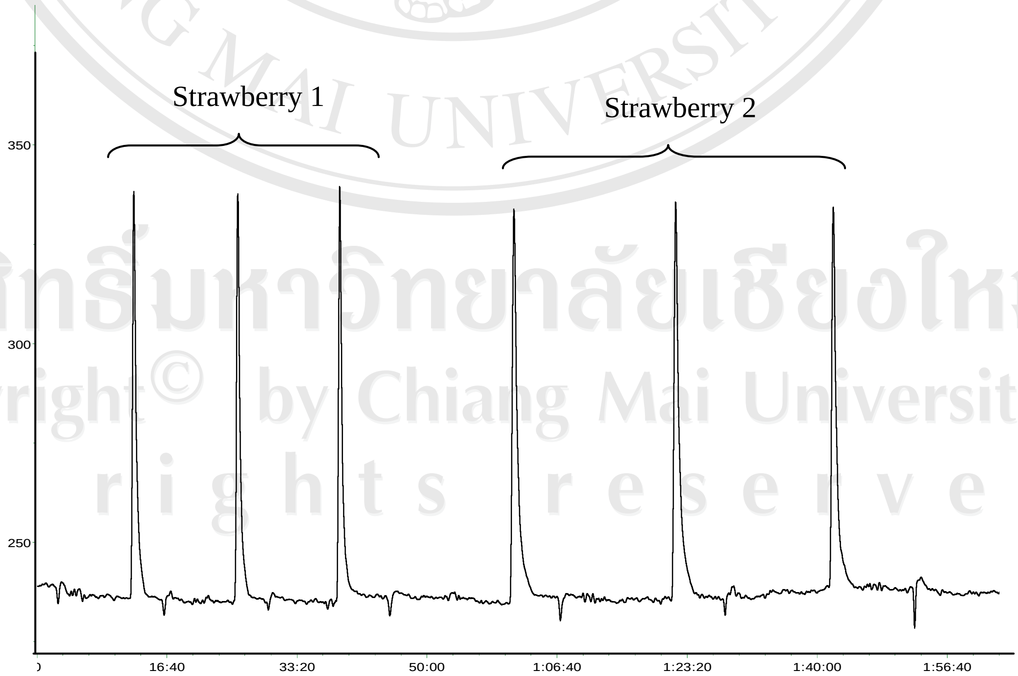

Figure 24 Stopped-FIA gram of strawberry samples

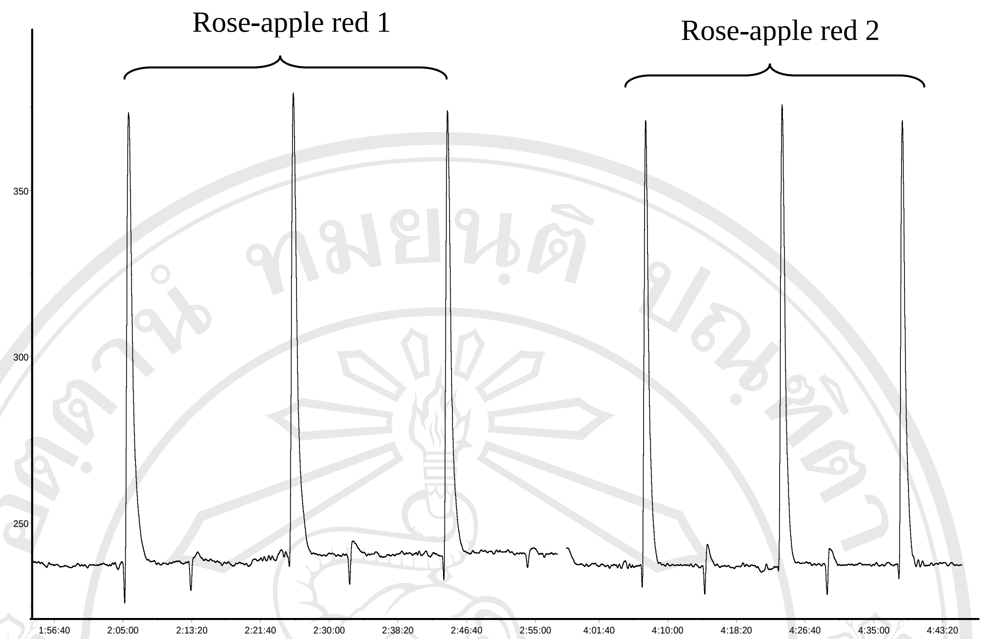

Figure 25 Stopped-FIA gram of rose-apple red samples

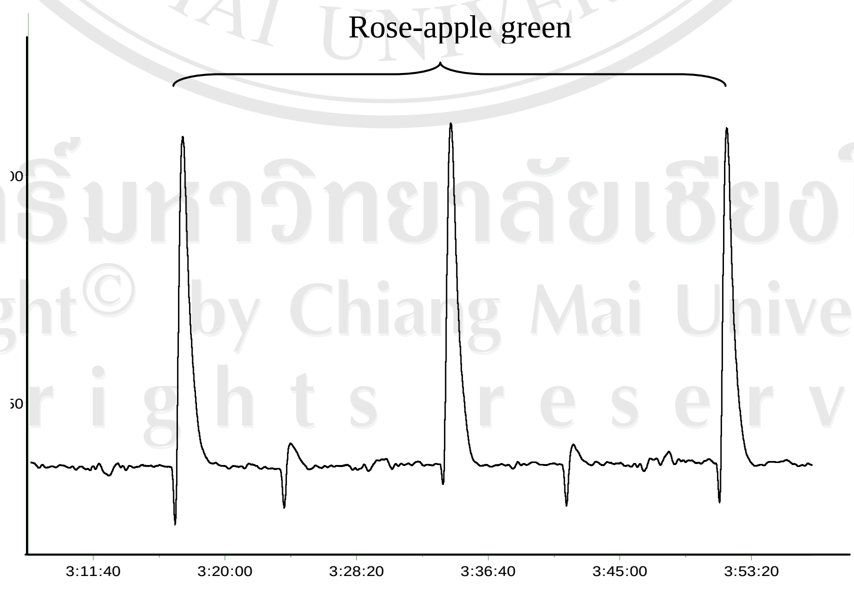

Figure 26 Stopped-FIA gram of rose-apple green sample

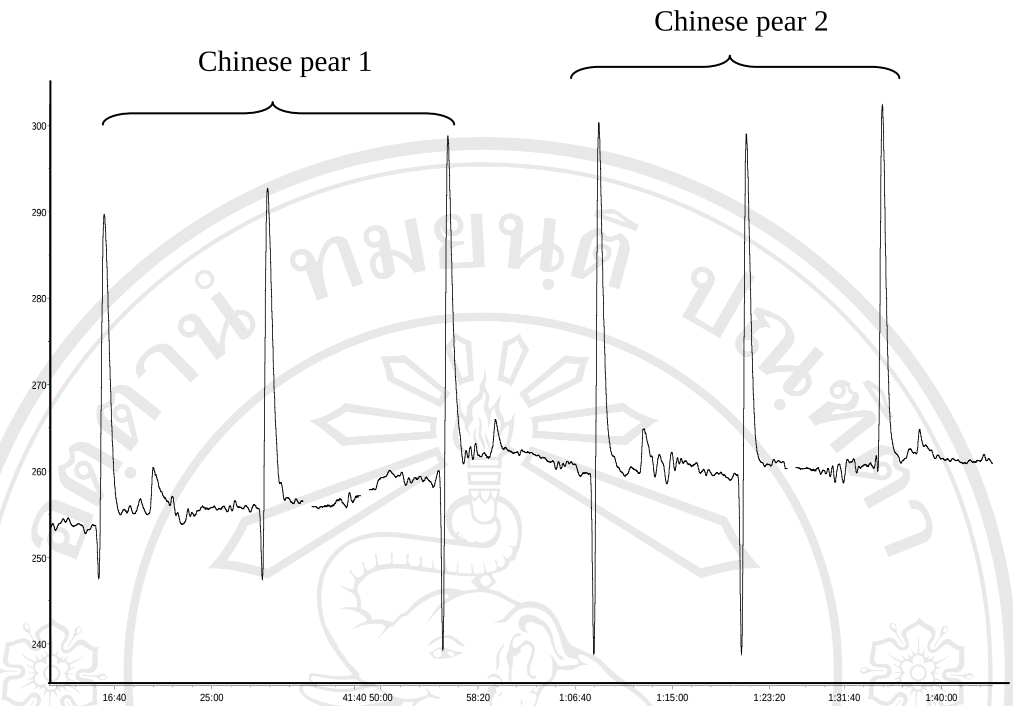

Figure 27 Stopped-FIA gram of Chinese pear samples

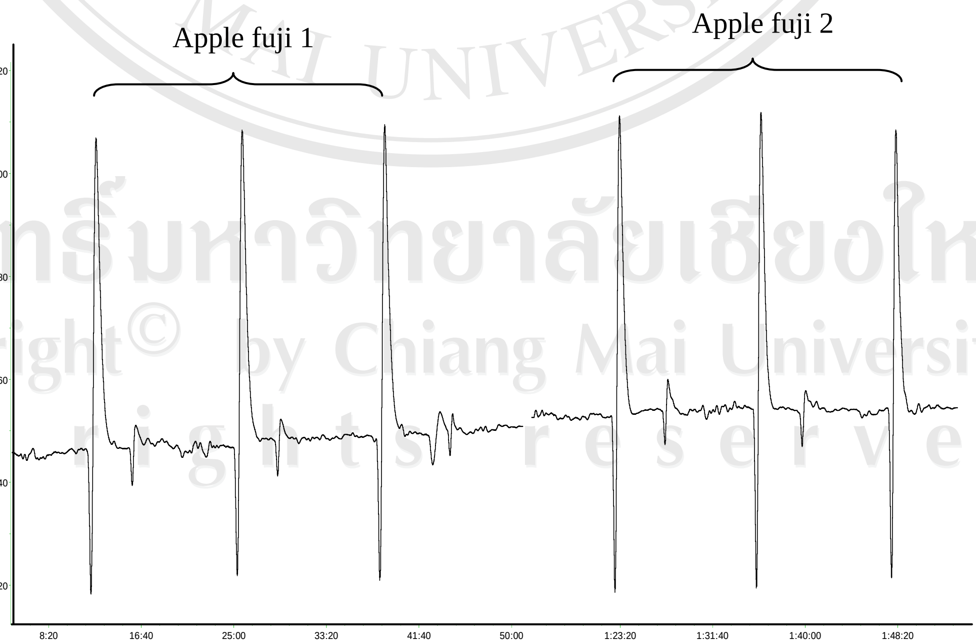

Figure 28 Stopped-FIA gram of apple fuji samples

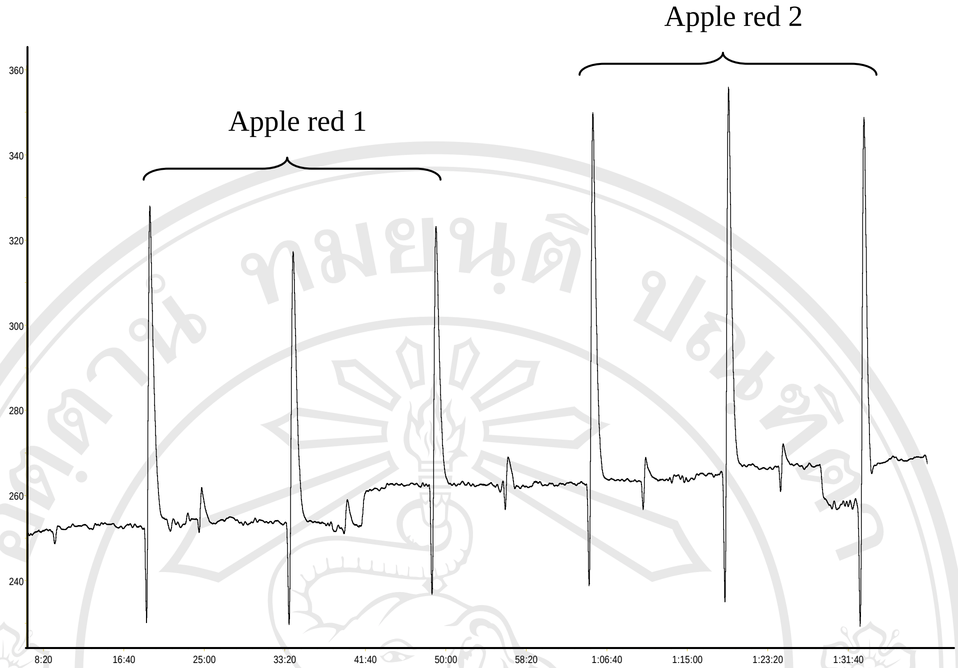

Figure 29 Stopped-FIA gram of apple red samples

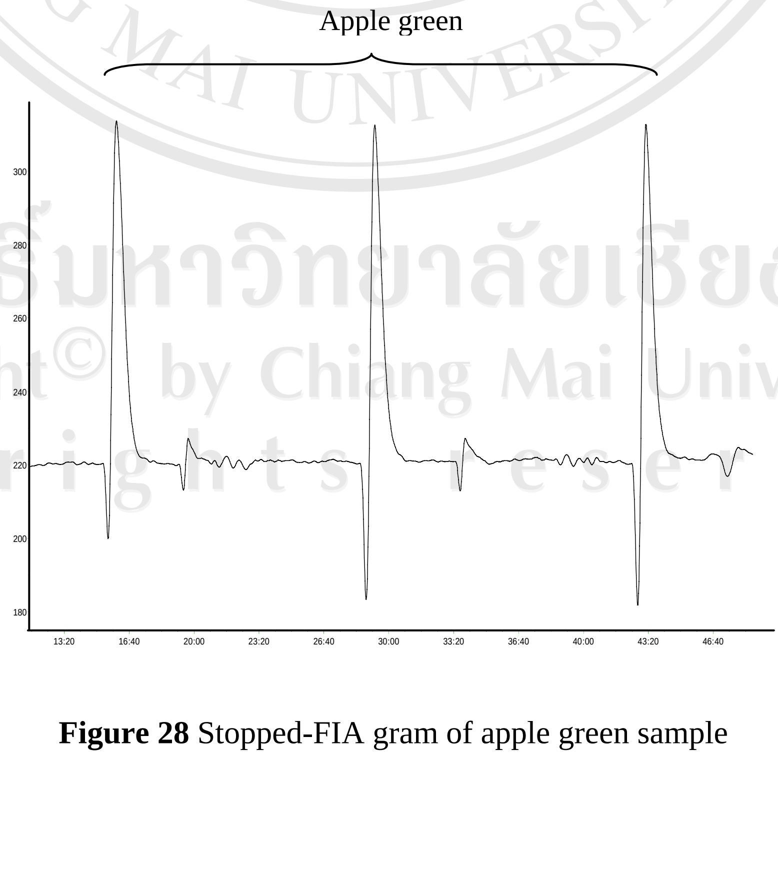

Figure 28 Stopped-FIA gram of apple green sample

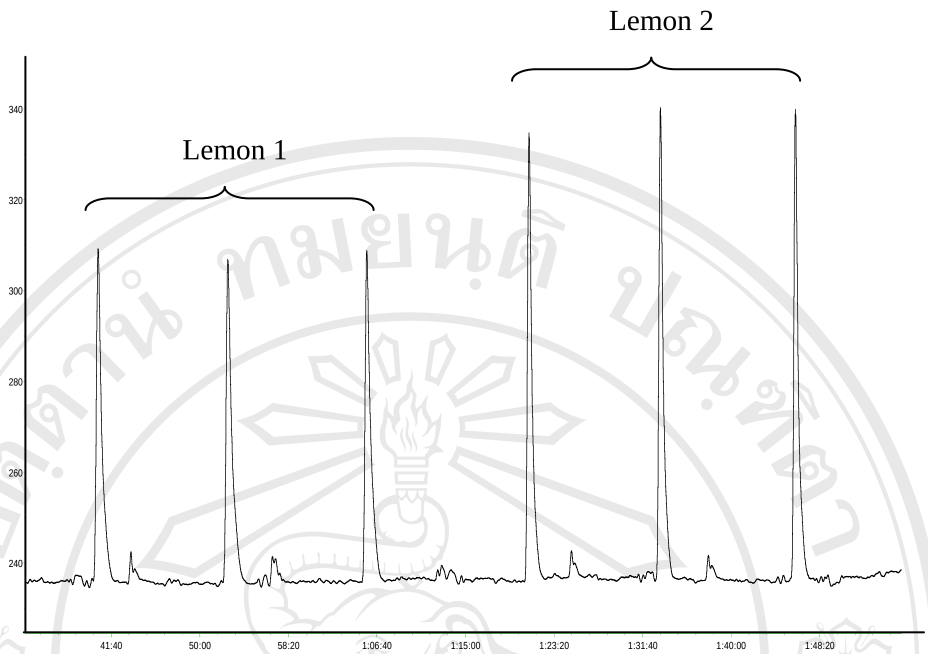

Figure 30 Stopped-FIA gram of lemon samples

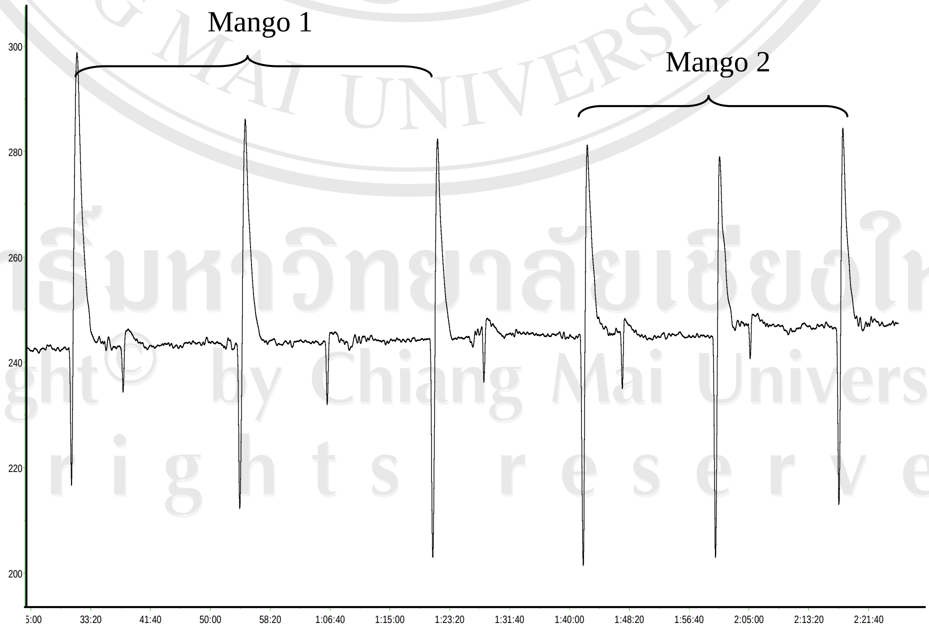

Figure 28 Stopped-FIA gram of mango samples

6. Effect of tartrate concentrations

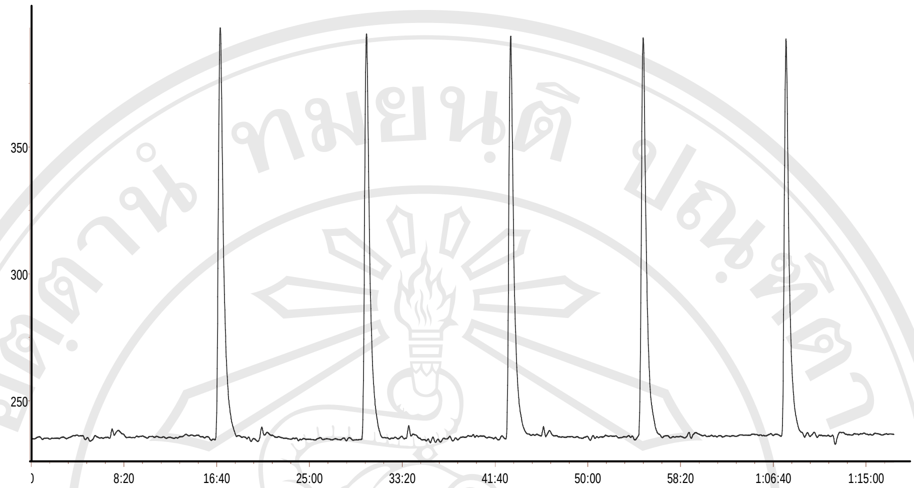

Figure 29 Stopped-FIA gram of ascorbic acid concentration at 60 mg/L

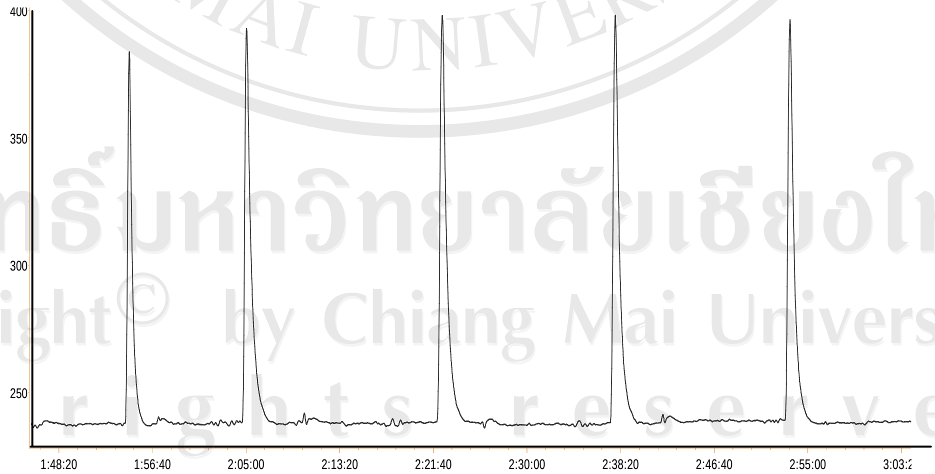

Figure 29 Stopped-FIA gram of ascorbic acid concentration at 60 mg/L

add tartrate concentration 60 mg/L

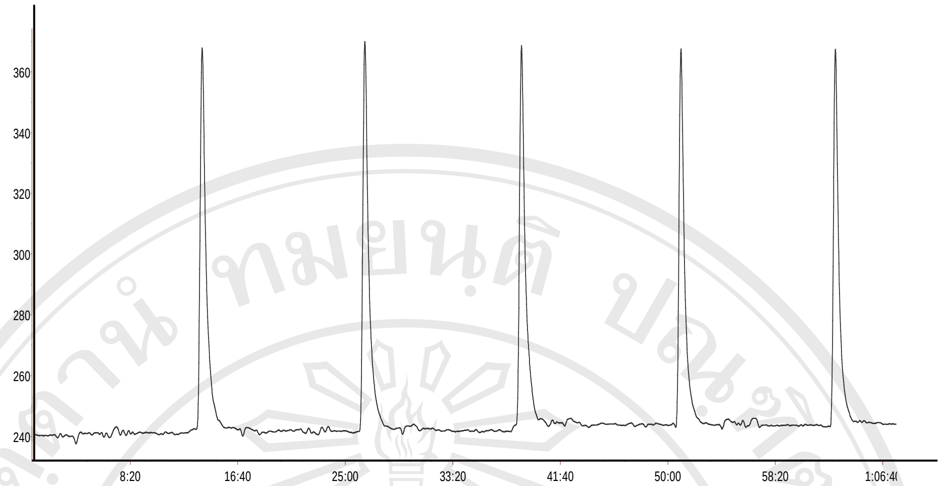

Figure 30 Stopped-FIA gram of ascorbic acid concentration at 60 mg/L add tartrate concentration 300 mg/L

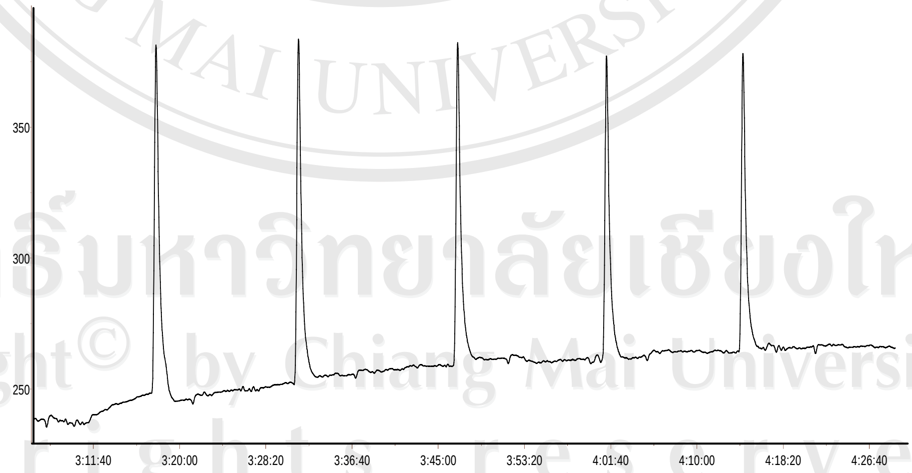

Figure 31 Stopped-FIA gram of ascorbic acid concentration at 60 mg/L add tartrate concentration 600 mg/L

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2007 Wiyarat Kumutanat[1], Wasin Wonkwilai[2], Kate Grudpan[1,2], Shoji Motomizu[3], Tadao Sakai[4] and Somchai Lapanantnoppakhun[1,2], Development of Stopped-Flow System for Ascorbic acid Determination (Oral and Poster Presentation), International Symposium on Flow Based Analysis VII 16-18 December 2007, Chiang Mai, Thailand.

2008 Wiyarat Kumutanat[1], Wasin Wonkwilai[2], Witsanu Jangbai[1], Kate Grudpan[1,2] and Somchai Lapanantnoppakhun[1,2], A simple macro-chip with flow based technique for ascorbic acid assay using a vanadium salt (Poster Presentation), Symposium for Younger Generation Researchers 29 August 2008, Chiang Mai, Thailand.

2008 Wiyarat Kumutanat[1], Wasin Wonkwilai[2], Kate Grudpan[1,2] and Somchai Lapanantnoppakhun[1,2], Rapid test for trans fatty acid (Poster Presentation), The 15th International Conference on Flow Injection Analysis (ICFIA2008), 28 September – 3 October 2008, Nagoya, Japan.

2008 Wiyarat Kumutanat[1], Wasin wongwilai [2], Krittiya Koonyotying[1], Kate Grudpan[1,2], Shoji Motomizu[3], Tadao Sakai[4] and Somchai Lapanantnoppakhun[1,2], Determination of vitamin C in some fruits using molybdenum salts by flow based techniques (Poster Presentation), The 15th International Conference on Flow Injection Analysis (ICFIA2008), 28 September – 3 October 2008, Nagoya, Japan.

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Vitamin C (ascorbic acid) is an antioxidant which is essential nutrient for human. Vitamin C deficiency can lead to a disease called scurvy which is characterized by abnormalities in the bones and teeth. Many fruits contain vitamin C and their juices are the main source of ascorbic acid for most people in Thailand. Various analytical instruments have been employed for analysis of vitamin C samples. But these devices are expensive and consume high amounts of chemicals.

The aim of this work is to apply stopped-flow injection analysis with the molybdenum blue method for determination of vitamin C in some fruit samples. The developed system offers simple and low cost devices with increase in sensitivity while consuming low amount of sample and reagent and producing less waste as compared to the titrimetric method. This developed system should be useful for routine determination of vitamin C in many sources such as foods, vegetables and pharmaceutical.