



**APPENDICES**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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**APPENDIX A****Standard method for determination of fumaric acid in fruit juice [6]****Preparation of reagents**

*Fumaric acid standard solution 500 mg/L:* Accurately weigh 0.5 g fumaric acid and transfer to 100 mL volumetric flask. Dilute to volume with methanol.

*Supporting electrolyte solution:* Dissolve 7.70 g tetramethylammonium bromide in 200 mL H<sub>2</sub>O to which has been added 0.210 g LiCl; shake vigorously and dilute to 500 mL with H<sub>2</sub>O

*Working standard fumaric acid solution:* Transfer 0.15, 0.5, 0.75 and 1 mL aliquots fumaric acid standard solution to five 25 mL volumetric flask that containing 4.85, 4.5, 4.25 and 4 mL methanol, respectively. Dilute to volume with supporting electrolyte solution.

**Preparation of sample assay solution**

Transfer 10 mL fruit juice to 25 mL volumetric flask containing 5 mL methanol. Dilute to volume with supporting electrolyte solution.

**Determination**

Transfer each of working standard solution or sample solution 10 mL to measuring cell. Purge N<sub>2</sub> gas three minute before measure current. Peak current is linear proportional to the fumaric acid concentration. Fumaric acid content in fruit juice can be evaluated from a calibration graph.



## APPENDIX C

### Standard titrimetric method for determination of ascorbic acid in fruit juice[67]

#### Preparation of reagents

*Extracing solution – Metaphosphoric acid-acetic acid solution:* Dissolve 15 g  $\text{HPO}_3$  pellets in 40 mL  $\text{CH}_3\text{COOH}$  and 200 mL  $\text{H}_2\text{O}$ ; dilute to 500 mL and filter rapidly through filter paper into glass-stoppered bottle.

*Ascorbic acid standard solution 1000 mg/L:* Accurately weigh 0.5 ascorbic acid and transfer to 100 mL volumetric flask. Dilute to volume with  $\text{HPO}_3\text{-CH}_3\text{COOH}$ .

*Indophenol standard solution:* Dissolve 0.1250 g 2,6-dichloroindophenol Na salt in 100 mL  $\text{H}_2\text{O}$  to which has been added 0.1050 g  $\text{NaHCO}_3$ ; shake vigorously, and when dye dissolves, dilute to 500 mL with  $\text{H}_2\text{O}$ . Filter through filter paper No.42 into amber glass-stoppered bottle. Keep out of direct sunlight, and store in refrigerator.

#### Preparation of sample assay solution

*Fruit and vegetable juices:* Mix thoroughly by shaking to ensure uniform sample, and filter through absorbent cotton. Add of 100 mL prepared juice to equal volume of  $\text{HPO}_3\text{-CH}_3\text{COOH}$  solution.

#### Determination

*Standardization:* Transfer three 2.0 mL aliquots ascorbic acid standard solution to each of three 50 mL Erlenmeyer flasks containing 5.0 mL  $\text{HPO}_3\text{-CH}_3\text{COOH}$  solution. Titrate rapidly with indophenol solution from 50 mL buret until light but distinct rose pink persists > 5 s. Similarly titrate 3 blanks compose of 7.0 mL  $\text{HPO}_3\text{-CH}_3\text{COOH}$  solution.

*Titration of juice sample:* Repeat the above titration, but substitute juice in place of the standard ascorbic acid solution.

## APPENDIX D

### Voltammetric method for determination of ascorbic acid in vitamin preparations[68]

#### Preparation of reagents

*0.5 M phosphate buffer solution if pH 6:* Dissolve 14.71 g  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  salt in 200 mL  $\text{H}_2\text{O}$  to which has been added 1.00 g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ; shake vigorously, and when salts dissolves, dilute to 1000 mL with  $\text{H}_2\text{O}$ . Keep in glass-stoppered bottle.

*Ascorbic acid standard solution 1000 mg/L:* Accurately weigh 0.5 ascorbic acid and transfer to 100 mL volumetric flask. Dilute to volume with  $\text{H}_2\text{O}$ .

*Working standard ascorbic acid solution:* Transfer 0.50, 1.25, 2.50, 3.75 and 5.00 mL aliquots ascorbic acid standard solution to five 25 mL volumetric flasks that containing 4.50, 3.75, 2.50, 1.25 and 0 mL  $\text{H}_2\text{O}$ , respectively. Dilute to volume with phosphate buffer solution.

#### Preparation of sample assay solution

Grind and weigh pharmaceutical tablet sample before dissolve to make 1000 mg/L stock solution. Transfer 2.5 mL aliquot to 25 mL volumetric flask containing 2.5 mL  $\text{H}_2\text{O}$ . Dilute to volume with phosphate buffer solution.

#### Determination

Transfer each of working standard solution or sample solution 10 mL to measuring cell. Purge  $\text{N}_2$  gas three minute before measure current. Peak current is linear proportional to the ascorbic acid concentration. Ascorbic acid content in fruit juice can be evaluated from a calibration graph.

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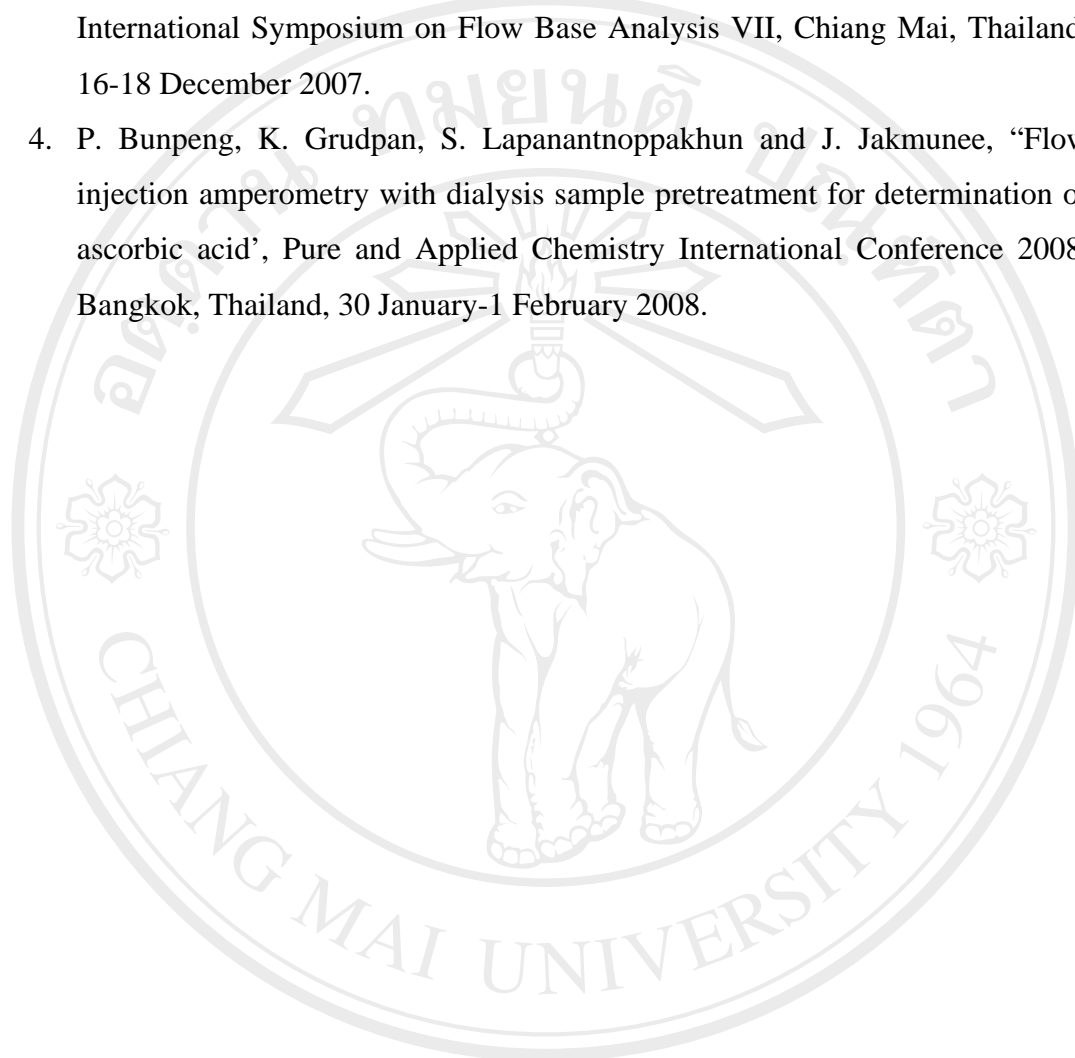
**List of Publications:**

P. Bunpeng, S. Lapanantnoppakhun, K. Grudpan and J. Jakmune, *Chiang Mai Journal of Science*, 2008, in press.

**List of Conferences:**

1. P. Bunpeng, K. Grudpan, S. Lapanantnoppakhun and J. Jakmune, "Flow Injection Amperometric Method for Determination of Ascorbic Acid in Fruit juices", The 6<sup>th</sup> Annual Symposium on TRF Senior Research Scholar and Research Group on Innovation on Analytical Instrumentation CHE, Chiang Mai University, Chiang Mai, Thailand, 16 August 2007.
2. P. Bunpeng, J. Jakmune and K. Grudpan, "Development of Flow Injection – Amperometric Method for Determination of Ascorbic Acid", 33<sup>rd</sup> Congress on Science and Technology of Thailand, Nakornsrihammarat, Thailand, 18-20 October 2007.

3. P. Bunpeng, K. Grudpan, S. Lapanantnoppakhun and J. Jakmune, "Flow injection amperometric detection for determination of ascorbic acid", International Symposium on Flow Base Analysis VII, Chiang Mai, Thailand, 16-18 December 2007.
4. P. Bunpeng, K. Grudpan, S. Lapanantnoppakhun and J. Jakmune, "Flow injection amperometry with dialysis sample pretreatment for determination of ascorbic acid", Pure and Applied Chemistry International Conference 2008, Bangkok, Thailand, 30 January-1 February 2008.



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