APPENDICES

APPENDIX A

Flow Injection analysis

Flow injection analysis (FIA) is based on the injection of a liquid sample into a moving continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a flow through detector that continuously records as a signal such absorbance, potential or other physical parameters.

Three principle of FIA are sample injection, controlled dispersion of the injected sample zone and reproducible timing of its movement from the injection point toward and into the detector. This permits obtaining a highly reproducible readout even when the mixing is incomplete, the chemistry may not reach equilibrium and the signal is transient [42].

The simplest flow injection analyzer is shown in Figure 1(a). That consists of a pump which is used to propel the carrier stream through a narrow tube. An injection port where a well-defined volume of a sample solution is injected into the carrier stream in a reproducible manner. A reactor or mixing coil is where the sample zone disperses and reacts with the components of the carrier stream, forming a species that is sensed by a flow-through detector and recorder. A typical recorder output has the form of a peak. The height and area of peak are generally evaluated their values are related to the concentration of the analyte. The shape of peak is mainly dependent on the physical process of dispersion of sample zone and the chemical process of the formation of the products, which occur at the same time [42].

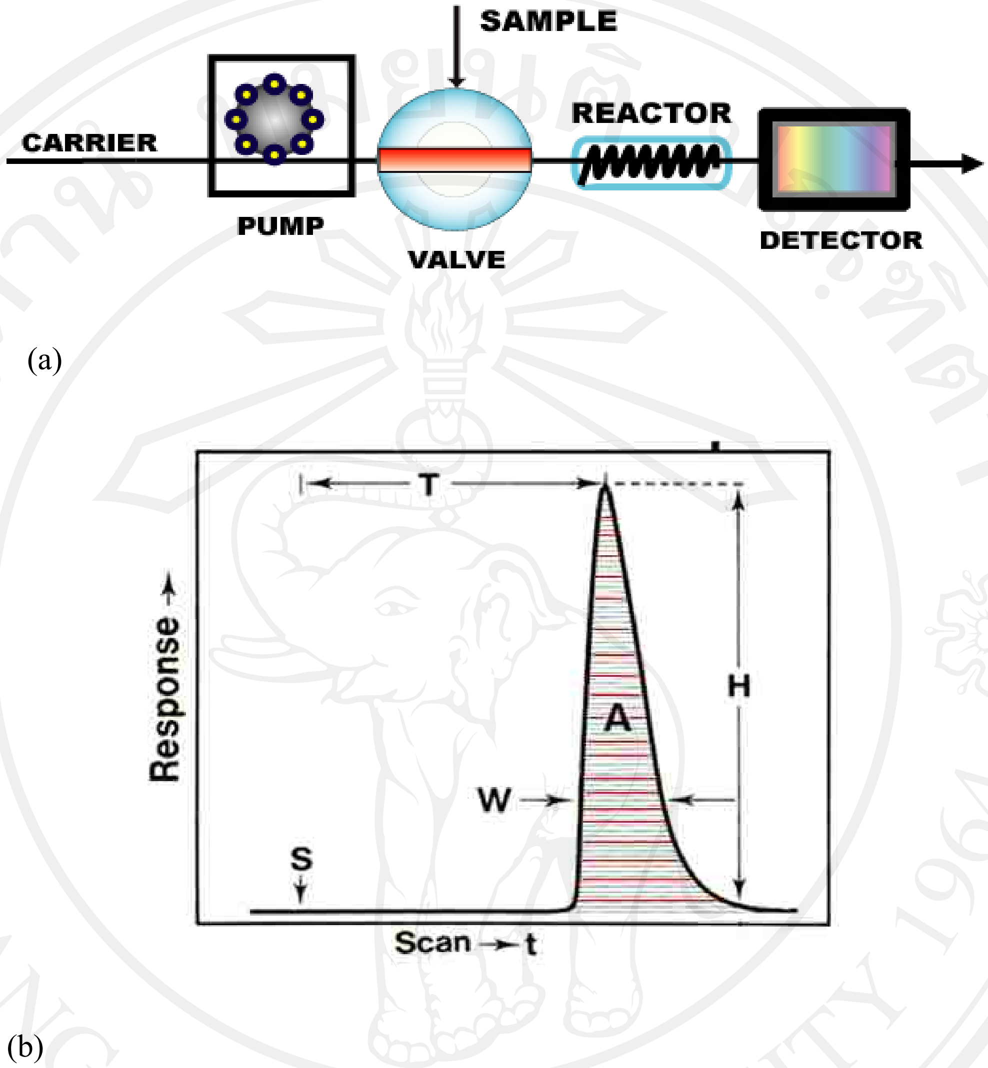


Figure 1 The simplest flow injection analyzer (a) and the FI-response (b) [42].

APPENDIX B

Amperometric method

1. Principle of amperometric method

Amperometric method is based on the measurement of current at a fixed potential. Either an oxidation or reduction is forced to occur by judicious selection of the potential applied to an electrode by a controlling potentiostat. The electrode acts as an oxidizing or reducing agent of variable power. In order to use amperometric measurements effectively, it is important to recognize that electrochemical detection is a surface technique, which means molecules not adjacent to the electrode must be moved to the surface to react. Flow injection analysis has been combined with amperometric detection. The various electrodes are placed in a flowing steam configured as a thin film. The film thickness is variable, with typical values being 15 – 125 µm [43]. The potential applied to the electrochemical cell between the reference and working electrode serves as the driving force for the detection redox reaction to occur. The working potential is usually determined by producing a current-potential plot. This is accomplished by monitoring the current at various potentials. At low positive (negative) potentials, the energy applied to the cell is insufficient. As the applied potential increases in the positive (negative) direction, the energy requirement for the reaction is now partially met, and a faradaic current ensues. As the potential is further increased, the faradaic current rises until a potential is reached, past which no further improvement in the current response is noted [43].

2. Instrumentation of flow injection amperometric system

The basic components of such a flow injection amperometric system is shown in Figure 1, which consist of a pump that provides a constant flow of carrier stream to an injection valve where the sample is introduced. The requirement of carrier stream must have low electrochemical activity that is low background current. A sample injection port may be manual or automated by should be of short durations. An injected sample may pass through a tube directly to the amperometric cell. A transducer cell is illustration in Figure 2. The thin-layer channel is defined by a gasket held between a stainless-steel block and a polymeric block is also. The stainless-steel block is the auxiliary electrode and provides a compartment for the reference electrode. The polymeric block contains the working electrodes. The potential selected by the user is applied between the reference and working electrodes while the current is passed between the auxiliary and working electrodes. Detection occurs at the working electrode in the thin-layer region. Electrodes of the same or different materials may be interchanged by simply swapping the working electrode half of the thin-layer cell. Glassy carbon and carbon paste have been used. This design allows easy collection of solute band without appreciable dispersion. Electrodes must have diameters of a few millimeters. Figure 2 also schematically depicts a thin – layer cell with two working electrodes, which can be arranged in a series or parallel configuration relative to a flow stream. A different potential can be applied to each electrode and the current of each monitored [43]

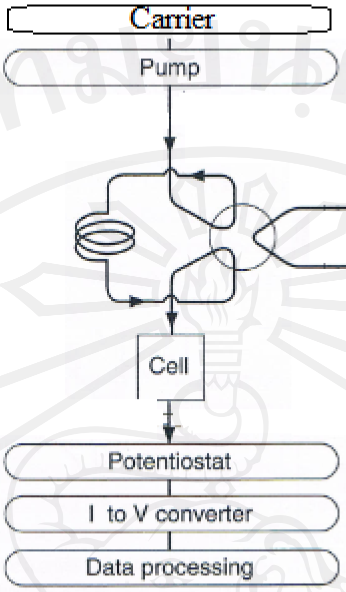


Figure 1 The basic components of flow injection amperometric system [43]

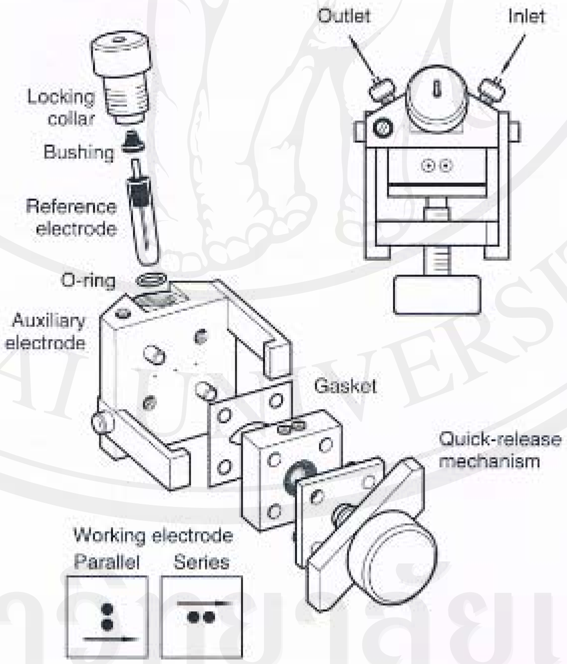


Figure 2 The amperometric cell [43].

APPENDIX C

FI – ferrous rartrate method

FI – ferrous tartrate method for estimation of antioxidant activity is shown in Figure 1. The system is comprised of peristaltic pump (Ismatec, Switzerland) with pump tubing, six port injection valve (Upchurch Scientific, USA), a mixing coil (PTFE, i.d. 0.5 mm), and a homemade flow through colorimeter. Sample or standard solution was injected into the carrier stream and mixed in-line with the reagent solution. The antioxidants react with ferrous tartrate solution leading to an increase in the absorbance of complex which could be detected by a flow through colorimeter at 540 nm and was the signal recorded as FIA peak. The reaction involved is shown in scheme 1. Antioxidative activity (as tannin equivalent) was calculated from a calibration equation, plotting between the peak height obtained and concentration of tannic acid.

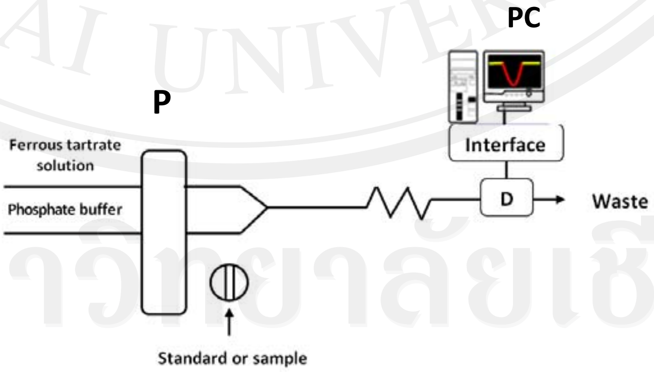
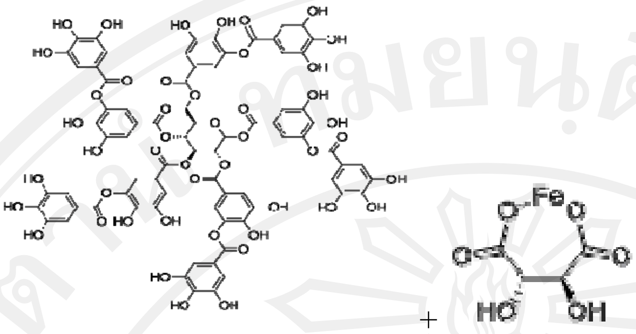


Figure 1 Manifold for estimation of antioxidant activity by FI – ferrous tartrate method. P = Pump, D = homemade flow through colorimeter, PC = Computer



→ Intense purple color complex

Scheme 1 Reaction between ferrous tartrate and tannin [21]

APPENDIX D

FI – colorimetric method based on FRAP reaction

FI – colorimetric method based on FRAP reaction for estimation of antioxidant activity is shown in Figure 1. The system consistes of a peristaltic pump (Ismatec, Switzerland) with pump tubing, a six-port injection valve (Ogawa, Japan) with injection loop 50 µL, a mixing coil (PTFE, i.d. 0.5 mm) and a homemade flow through colorimeter. The system was controlled by a personal computer using a software program written in-house. Sample or standard solution was injected into the carrier stream and mixed in-line with the reagent solution. The antioxidants react with Fe(III) to produced Fe (II) leading to an increase in the absorbance of Fe(II) - 1,10-phenanthroline complex which could be detected by a colorimeter with green LED as light source and the signal was recorded as FIA peak. Antioxidative activity (as ascorbic acid equivalent) was calculated from a calibration equation, plotting between the peak height obtained and concentration of ascorbic acid.

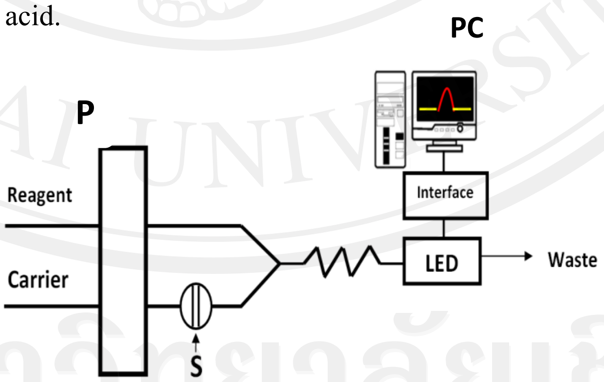
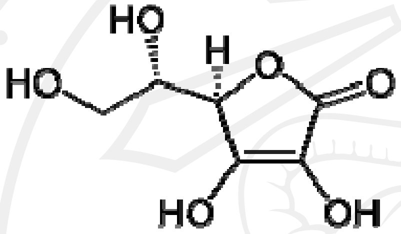


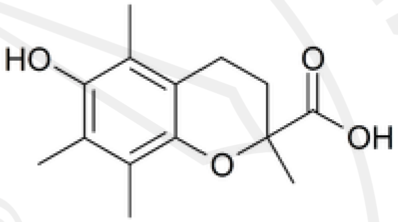
Figure 1. Manifold of flow injection colorimetric method based on FRAP reaction for estimation of antioxidative activity. S = standard/sample, Carrier = Deionized water and Reagent = 1.5 mM Fe(III) – 1.5 mM 1,10-phenanthroline.

APPENDIX E

Structure of some antioxidant compounds

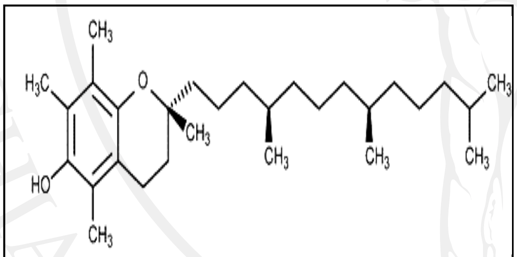


Ascorbic acid [44]

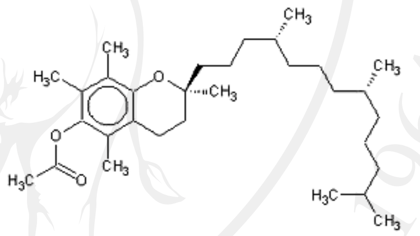


Trol

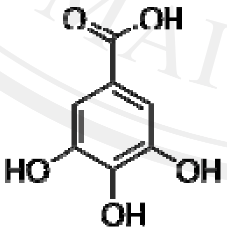
ox [45]



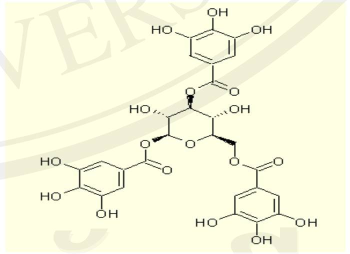
α-Tocopherol [46]



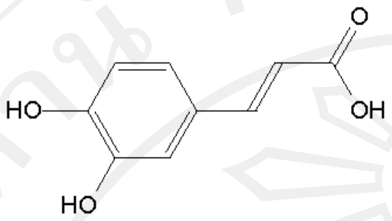
α-Tocopherol acetate [47]



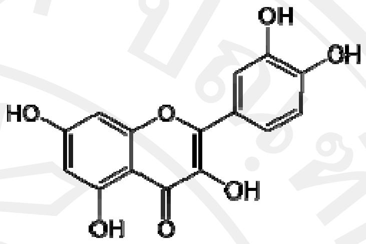
Gallic acid [48]



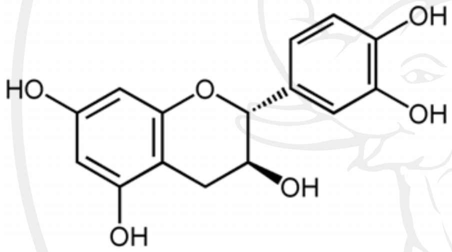
Gallic acid [49]



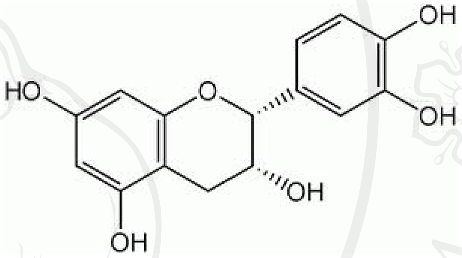
Caffeic acid [50]



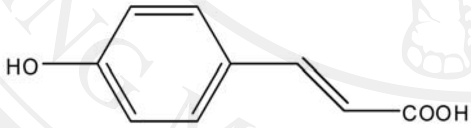
Quercetin [51]



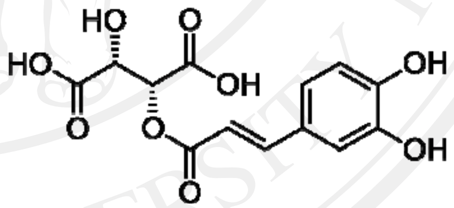
Cetechin [52]



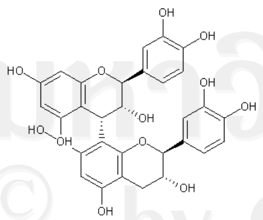
Epicetechin [53]



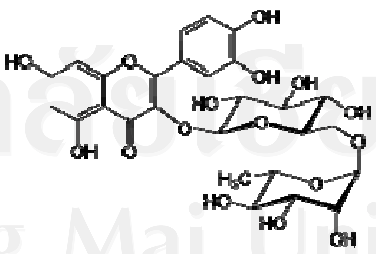
p – Coumaric acid [54]



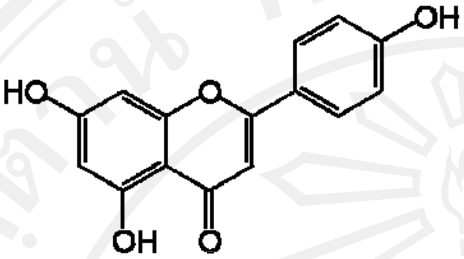
Caftaric acid [55]



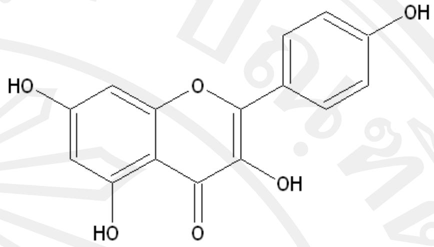
Procyanidin B2 [56]



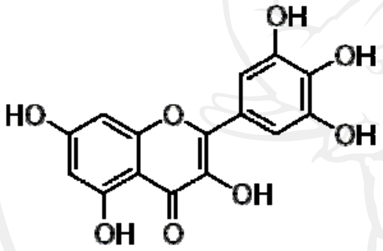
Rutin [57]



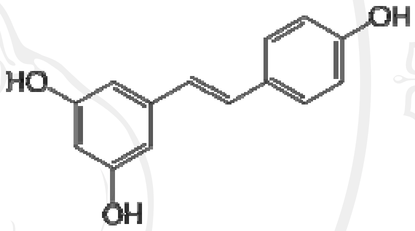
Apigenin [58]



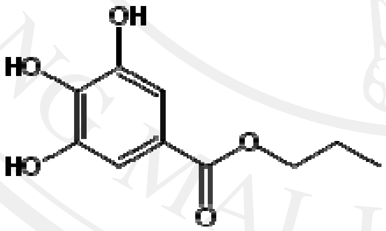
Kaempferol [59]



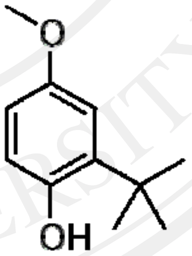
Myricetin [60]



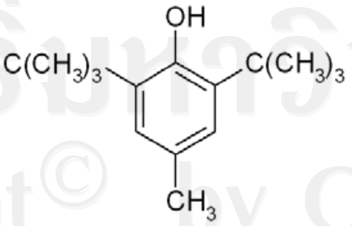
trans - Resveratrol [61]



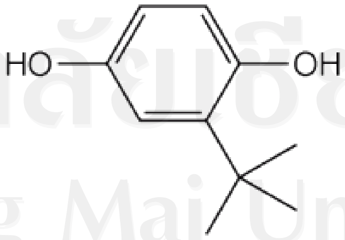
Propyl gallate [62]



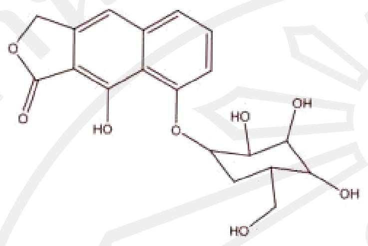
Butylated hydroxyanisole [63]



Butylated hydroxytoluene [64]



Tert-Butylhydroquinone [65]



Geshoidin [66]