Thesis Title	Development of flow injection analysis system for the determination of antioxidant capacity in some natural extracts
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ABSTRACT

A flow injection (FI) spectrophotometric system was developed for the determination of antioxidant capacity. The analysis is based on the color disappearance due to the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by antioxidant compounds. Butylated hydroxy toluene (BHT) and ascorbic acid were used as antioxidant standards. The proposed FI system was a single-line system consisted of a peristaltic pump, a 6-port injection valve with a 50 μL sample loop, a reaction coil and a visible spectrophotometer (wavelength = 520 nm). $DPPH^{\bullet}$ reagent was pumped through the reaction coil and detector. A standard/sample was injected into the $DPPH^{\bullet}$ stream and mixed at the reaction coil. The product zone was finally detected in the spectrophotometer. Effects of the $DPPH^{\bullet}$ concentration (0.025-0.150 mM), $DPPH^{\bullet}$ flow rate (0.5-2.0 mL/min), and reaction coil length (50-500 cm) were studied. The optimum conditions were $DPPH^{\bullet}$ concentration 0.075 mM, $DPPH^{\bullet}$ flow rate 1 mL/min and reaction coil length 200 cm. The optimized system provided the linear range of 0.5-15.0 mM BHT and 0.010-0.300 mM ascorbic acid with correlation coefficient (R^2) of 0.9994 and 0.9995, respectively. %RSD (n=10) was less than 5%. Detection limit and quantitation limit were 0.252 mM and 0.409 mM for BHT and 0.008 mM and 0.017 mM for ascorbic acid, respectively. Sample throughput was 20 samples/hr. Validation of the FI method using BHT and ascorbic acid as standards was performed by comparing the antioxidant capacity

obtained by the FI method with the batch method. The results obtained by the FI method using ascorbic acid as standard agreed with those obtained by the batch method (*t*-test, confidence level 95%). However, the variance of the results obtained by the FI method using BHT as standard was significantly different from those obtained by using the batch method. The proposed FI method was probably able to apply to the analysis of fast reacting antioxidants. Using the FI-spectrophotometric system with ascorbic acid as standard the antioxidant capacity found in the natural sample extracts, vitamin E, commercial antioxidant product and commercial herbal products were 0.02-17.38 mM AAE, 0.01 mM AAE, 0.03 mM AAE, and 0.02-0.19 mM AAE respectively.

Keywords: 2,2-diphenyl-1-picrylhydrazyl (DPPH)/Antioxidant capacity/BHT/Ascorbic acid/Natural extracts/Flow injection analysis