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Flow Injection Analysis System for the Determination of Total Phenolic Compounds by Using Folin-Ciocalteu Assay

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Abstract: A simple, rapid and reliable flow injection analysis system for the determination of total phenolic compounds using the Folin-Ciocalteu method was established. The detection method was based on the reduction of a mixture of phosphotungstic and phosphomolybdic acid (Folin-Ciocaltue reagent) to tungsten and molybdenum oxide by phenolic compounds in the basic media and subsequent formation of a blue color product. The standard or sample solutions were injected into a carrier stream (distilled water) to react with a folin ciocaltue reagent and sodium carbonate to give the blue color product which was detected by spectrophotometer at 765 nm. The experimental conditions such as sample volume, flow rate of carrier and reagents, length of reaction coils and concentration of reagents were optimized. The relative standard derivation, RSD (20 replicates) of 5 ppm gallic acid was 0.72% with the detection limit (3S/N) of 0.0231 mg L^{-1}. A good linear calibration curve in the range of 0.5-100.0 mg L^{-1} was obtained with regression equation $y = 0.0123x + 0.021$, $R^2 = 0.9991$. The sampling throughput was 32 samples per hour. The effect of potential interferences such as citric acid, fructose and others were examined. The proposed method was successfully applied for the determination of the total phenolic compounds in tea.

Key words: Total phenolic compounds, antioxidant, flow injection analysis, spectrophotometry, Folin-Ciocalteu

INTRODUCTION

Phenolic compounds are a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic hydrocarbon group. They are diverse in structure but are characterized by hydroxylated aromatic rings (e.g., flavan-3-ols) and categorized as secondary metabolites. Furthermore, phenolic compounds may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyl fatty acids, sterols and glucosides. Phenolic compounds are plant metabolites widely spread throughout the plant kingdom. Some phenolic compounds in plants are polymerized into larger molecules such as the proanthocyanidins (PA or condensed tannins) and lignins. Their function in plants is often poorly understood. Recent interest in phenolic compounds stems from their potential protective role, through ingestion of fruits and varieties indigenous vegetables such as apple (Michalowski and Halaburda, 2001), black caraway, carrot, cranberry, heap, orange (Assimopoulou *et al*., 2006), tomato (Chang *et al*., 2006) against oxidative damage diseases (arteriosclerosis, cardiovascular, aging, coronary heart disease, stroke and

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cancers) (Tanizawa *et al.*, 1992; Aruoma, 1998; Abe and Berk, 1998; Abdi and Ali, 1999) essential for the growth and reproduction of plants, produced as a response for defending injured plants against pathogens.

Significantly, phenolic compounds have high antioxidant capacity, are excellent free radical scavengers (Gaulejac *et al.*, 1999) and have been used in processed foods in recent years. Several analytical procedures have been reported in literature for determining total phenolic compound such as the Jerumanis method (Schoonen and Sales, 2002) liquid chromatography (Guillen *et al.*, 1996; Ho *et al.*, 1999; Vinas *et al.*, 2000; Tial *et al.*, 2005) electrophoretic (Cartoni *et al.*, 1995; Peing *et al.*, 2004) spectrophotometric with Diode Array Detection (DAD) (Oszmianski *et al.*, 2007) and Florimetric Detection (Schoonen and Sales, 2002).

The generally accepted reaction for the determination of total phenolic compounds is the Folin-Ciocalteu assay. All of these methods however are inconvenient, stepwise and time-consuming. Recently, Flow Injection Analysis (FIA) was chosen as an alternative quantitative analysis. FIA was established in recent decades and has several advantageous features including simple, sensitivity, versatility, rapid, convenient, high precision, high accuracy and low cost. Recently, Gonzalez-Rodríguez *et al.* (2002) developed a simultaneous flow injection approach-a diode array spectrometer -for monitoring and determination of total polyphenol and anthocyanidine in red wines. Schoonen and Sales (2002) applied the photometric FIA for determination of polyphenol in wines based on the Jerumanis method. A sequential injection analysis method was developed for the determination of the total polyphenol index in wines (Moreno *et al.*, 2004) based on the Folin-Ciocalteu reaction, which is monitored spectrophotometrically. The on-line fraction of the polyphenols content in wines was reported by Lucena *et al.* (2005). The sample solutions were isolated from the matrix by solid-phase extraction of RP-C_{18}. An Evaporative Light Scattering Detection (ELSD) was used as the detection system. Average repeatability, expressed as relative standard deviation, was 4%.

In this study a simple and rapid FI spectrophotometric system for the determination of the total phenolic compounds was assembled utilizing Folin-Ciocalteu as reagent and applied to determine total phenolic compounds in tea samples.

MATERIALS AND METHODS

The experiment was conducted in October 2007 to April 2008. Tea samples were purchased at local market in Maha Sarakham province located in Northeastern of Thailand.

Apparatus

UV-VIS Spectrophotometer

For absorption spectra and absorbance measurement, a lambda-25 double beam UV-VIS spectrophotometer (Perkin Elmer, USA) furnished with a 1.0 cm Quartz cell was used and absorption spectra were scanned from 200 to 800 nm. The spectra data was analyzed by Version 2.85.04 software (Perkin Elmer, USA).

Flow Injection Analysis System

A schematic diagram for the proposed flow injection analysis system is presented in Fig. 1. A peristaltic pump (Perkin Elmer, FIAS300, USA) was used for propelling the Carrier Solution (CS) and reagent (RS1, RS2). Solinoid injection valve, I, with a sample loop (200 μL) was used for introducing gallic acid as standard solutions, as well as sample solutions, S, into carrier stream. PTFE tubing (i.d.= 0.89 mm) was used for flow lines. The absorbance was measured with UV-Visible spectrophotometer (Perkin Elmer Lampda Bio-40, USA). A personal computer with FIA monitor data processing software (Perkin Elmer, USA) was used for controlling the apparatus and recording data.

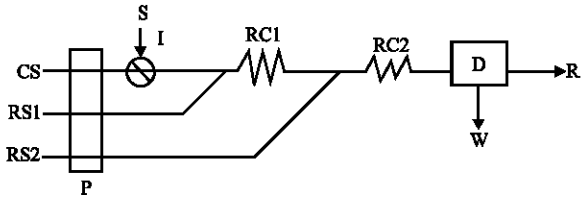

Fig. 1: Flow diagram for the determination of total phenolic compounds, S, standard (gallic acid) solution or sample solution; CS, carrier (double distilled water); RS1, 0.2 M of folin ciocalteu reagent solution; RS2, 75 g L^{-1} of Na_2CO_3 solution; P, peristaltic pump; RC1 and RC2, reaction coils; I, injection value; D, detector; W, waste; R, recording system

Reagents

All reagents used were of analytical grade and double distilled water was used for the preparation of all solutions. The 1000 mg L^{-1} of gallic acid (Fluka, Switzerland) stock solution was prepared by using double-distilled water. The calibration curve was prepared by diluting gallic acid solutions from 0.5 to 100 ppm from stock solutions. A 75 g L^{-1} sodium carbonate (Univar, Australia) and 0.2 M Folin-Ciocalteu reagent (BHD, England) were also prepared in double-distilled water. Sample solutions were prepared by soaking 1 g of tea powder samples in hot double-distilled water for about 10 min. Then, the tea solution samples were filtrated and adjusted to a 100 mL volumetric flask prior to injection into the FIA system. The following compounds were used for interference testing; sodium chloride, citric acid, sucrose, fructose and sodium bisulfite. All these chemicals were purchased from BHD, England.

Procedure

Flow Injection Analysis Methods

In Fig. 1, each flow rate of all solutions was set at 1.9 mL min^{-1}. The sample solutions or standard, gallic acid solution were injected into carrier stream and then merged with the Folin-Ciocalteu reagent stream and reacted continuously in 400 mm of 0.89 mm i.d. reaction coil (RC1). After that, a flowing solution was merged with the sodium carbonate solution and flowed into a 5000 m of 0.89 mm i.d. reaction coil (RC2). The absorbance changes of reaction of the blue color product were measured by UV-VIS spectrophotometer at 765 nm. The flow signals were recorded by personal computer and the concentration of the phenolic compounds in tea samples was calculated.

Spectrophotometric Method

The content of the total phenolic compounds was determined by Folin-Ciocalteu assay. Aliquots of standard gallic acid solution or 1 mL of test samples solution were mixed with 5 mL of 0.2 M Folin-Ciocalteu reagent and 4.0 mL of 75 g L^{-1} sodium carbonate. The blue color product was measured at 765 nm after standing at room temperature for 30 min. Total phenol content was expressed as mg gallic acid equivalents/g lettuce (mg GAE/g).

RESULTS AND DISCUSSION

Selection of Detection Wavelength

Gallic acid as standard solution was prepared according to the standard procedure and the absorption spectra was obtained in the range from 400 to 800 by a spectrophotometer. The maximum absorption wavelength of the blue color product was 765 nm as shown as Fig. 2.

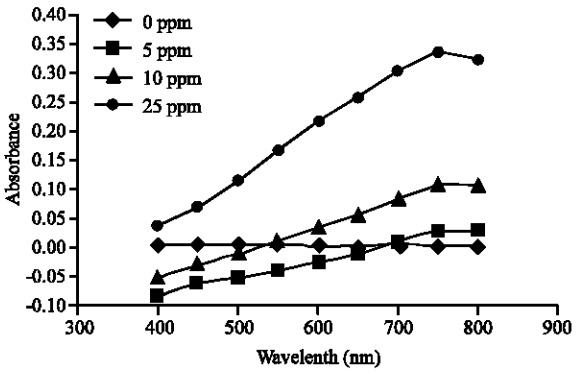

Fig. 2: Absorption spectrum of the blue color complex from 5 ppm gallic acid as standard solution and 0.2 M Folin ciocalteu reagent in basic media (75 g L^{-1} Na_2CO_3) after standing at room temperature for 30 min

Experimental Variable for FIA System

The effect of a sample injection volume in the range of 50 to 500 μL was investigated. It can be seen that the absorbance increased with an increase of sample volume up to 300 μL, beyond which the highest absorption remained constant (Fig. 3a). A sample injection volume of more than 300 μL caused the broad peak and used analysis time. Thus, a sample injection volume of 300 μL was selected for further study.

The effect of flow rate of carrier (double-distilled water, CS) and reagent solutions, which was Folin-Ciocalteu reagent (RS1) and sodium carbonate (RS2), was studied in the range of 0.8 to 2.4 mL min^{-1} which indicate that the sensitivity slightly increased with an increase of flow rate up to 1.9 mL min^{-1}. At faster flow rates, the signals decreased. Therefore, a 1.9 mL min^{-1} rate was chosen for further experiment (Fig. 3b).

The effect of Reaction Coil Length (RC1) was also examined in the range of 100 to 1600 mm. The absorbance was almost constant when the 100-800 mm reaction coil length was used. By using longer reaction coil length, the signals decreased, because too large a dispersion occurred and broad peaks were obtained. The reaction coil length of 300 mm was chosen for rapid analysis measurement (Fig. 3c).

The effect of reaction coil length, RC2 was investigated in the range of 1000 to 8000 m. The results obtained are shown in Fig. 3d. The absorbance increased with an increase of the length of the reaction coil up to 5000 mm. Therefore, 5000 mm was used for further study.

The effect of concentration of Folin-Ciocalteu solution was examined in a range from 0.02 to 1 M. The results obtained are shown in Fig. 3e. It can be seen that the absorbances become maximal and almost constant when the concentration of Folin-Ciocalteu solution was more than 0.2 M. In further experiments, 0.2 M Folin-Ciocalteu solution was selected.

The effect of concentration of sodium carbonate solution was studied from the range of 5 to 150 g L^{-1}. The absorbance increased with increasing concentration of sodium carbonate solution. However, a concentration of sodium carbonate higher than 75 g L^{-1} caused precipitation to occur. The suitable concentration of sodium carbonate solution was 75 g L^{-1} for optimum FIA system.

Analytical Characteristics

In this study, the standard solutions of gallic acid were used for preparing the calibration curve at the concentration in the range from 0.5 to 100 mg L^{-1}. The equation of calibration graph was expressed as $Y = 0.0123X + 0.021$, where Y was absorbance and X was gallic acid concentration, with a correlation coefficient of 0.9991. The Relative Standard Deviation (RSD) was 0.072% for 5 mg L^{-1}

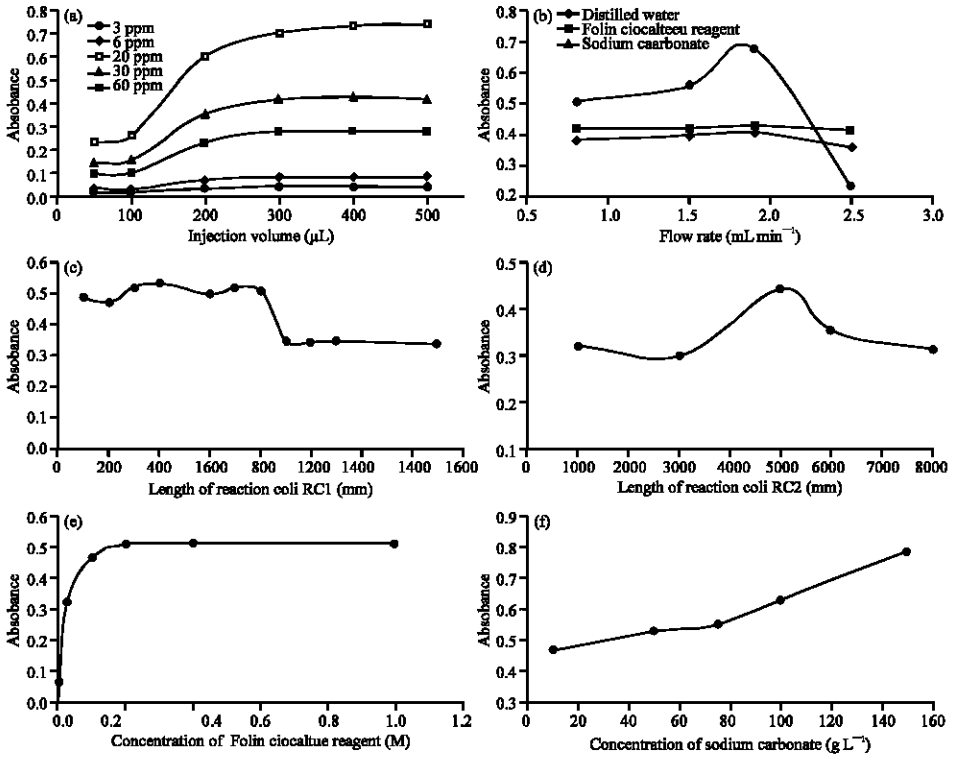

Fig. 3: Absorbance from analysis of standard solution of gallic acid at concentration 30 ppm recorded under different conditions, (a) different sample injection volume, (b) different flow-rate, (c) different length of reaction coil (RC1), (d) different length of reaction coil (RC2), (e) different concentration of folin ciocalteu reagent and (f) different concentration of sodium carbonate solution

Table 1: Interferences of FIA determination of total polyphenols (20 ppm gallic acid)

Interference species	Tolerance concentrations (mg mL^{-1})*
Citric acid	43.96
Fructose	7.84
Sucrose	31.99
Sodium bisulfite	4.96×10^{-3}
Sodium chloride	6.95

*Defined as less than ±5% relative error

(n = 20). The limit of detection (LOD) was 0.0213 mg L^{-1} (calculated from 3 times S/N). Thirty-two throughout samples per hour were obtained. The investigation interference species was conducted with regard to possible chemical interferences. The results are shown in Table 1, where the tolerable concentration is defined as the concentration of species causing less than ±5% relative error. The results revealed no significant interference from citric acid, fructose, sucrose, sodium bisulfite and sodium chloride.

Application of the Proposed Method to Real Samples

The proposed method was applied to the determination of the total phenolic compounds in 5 tea sample powders and compared with the batch-wise method shown in Table 2. It was found that the proposed and standard method have good agreement with a correlation coefficient of 0.9966 as shown in Fig. 4.

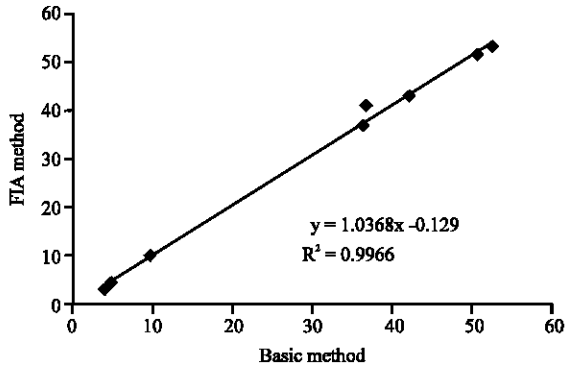

Fig. 4: Relationship between constants of total phenolic compounds in tea samples by using FIA method and batchwise method (spectrophotometric method)

Table 2: Total phenolic compound contents in tea samples by using the proposed FIA method and by batchwise method

	Total phenolic compound (expressed GA, mg G^{-1})	
Sample	Batchwise method (n = 3)	The proposed method (n = 3)
Tea 1	51.51±0.09	51.61±0.13
Tea 2	52.68±1.79	53.20±0.18
Tea 3	42.21±0.24	42.90±0.07
Tea 4	36.64±0.17	37.21±0.03
Tea 5	36.93±0.34	40.84±0.14
Tea 6	4.59±0.10	4.38±0.02
Tea 7	9.58±0.17	10.24±0.01
Tea 8	4.00±0.17	3.33±0.01
Tea 9	3.94±0.12	3.49±0.01

CONCLUSIONS

In the present study, a simple rapid and reliable flow injection system for the determination of the total phenolic compounds in teas was developed. The LOD of the proposed method was 0.0213 mg L^{-1} and the interferences of certain sugars, salt and organic acids commonly present in tea were negligible.

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REFERENCES

Abdi, S. and A. Ali, 1999. Role of ROS modified human DNA in the pathogenesis and etiology of cancer. Cancer Lett., 142: 1-9.

Abe, J. and B.C. Berk, 1998. Reactive oxygen species as mediators of signal transduction in cardiovascular disease. Trends Cardiovas. Med., 8: 59-64.

Aruoma, O.I., 1998. Free radical, oxidative stress and antioxidant in human health and disease. J. Am. Oil Chem. Soc., 75: 199-212.

Assimopoulou, A.N., D. Boskou, M.A. Anagnostopoulou, P. Kefalas and V.P. Papageorgiou, 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). Food Chem., 94: 19-25.

Cartoni, G., F. Coccioli and R. Jasionowska, 1995. Capillary electrophoretic separation of phenolic acids. J. Chromatogr. A, 709: 209-214.

Chang, C.H., H. Lin, C.Y. Chang and Y.C. Liu, 2006. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. J. Food Eng., 77: 478-485.

Gaulejac, N.S., Y. Glories and N. Vivas, 1999. Free radical scavenging effect of anthocyanins in red wines. Food Res. Int., 32: 327-333.

Gonzalez-Rodrýguez, J., P. Perez-Juan and M.D. Castro, 2002. Method for the simultaneous determination of total polyphenol and anthocyan indexes in red wines using a flow injection approach. Talanta, 56: 53-59.

Guillen, D.A., C.G. Barroso and J.A. Perez-Bustamante, 1996. Selection of column and gradient for the separation of polyphenols in sherry wine by high-performance liquid chromatography incorporating internal standards. J. Chromatogr. A, 724: 117-124.

Ho, P., T.A. Hogg and M.C.M. Silva, 1999. Application of a liquid chromatographic method for the determination of phenolic compounds and furans in fortified wines. Food Chem., 64: 115-122.

Lucena, R., S. Cardenas, M. Gallego and M. Valcarcel, 2005. Autoanalyzer for continuous fractionation and quantitation of the polyphenols content in wines. J. Chromatogr. A, 1081: 127-131.

Michalowski, J. and P. Halaburda, 2001. Flow-injection chemiluminescence determination of epinephrine in pharmaceutical preparations using raw apple juice as enzyme source. Talanta, 55: 1165-1171.

Moreno, C.L., P.C. Rudner1, J.M. Garcýa and J. Pavon, 2004. Development of a sequential injection analysis device for the determination of total polyphenol index in wine. Microchim. Acta, 148: 93-98.

Oszmianski, J., A. Wojdylo, E. Lamer-Zarawska and K. Swiader, 2007. Antioxidant tannins from rosaceae plant roots. Food Chem., 100: 579-583.

Peing, Y., Q. Chu, F. Liu and J. Ye, 2004. Determination of phenolic constituents of biological interest in red wine by capillary electrophoresis with electrochemical detection. J. Agric. Food Chem., 52: 153-156.

Schoonen, J.W. and M.G.F. Sales, 2002. Determination of polyphenols in wines by reaction with 4-aminoantipyrine and photometric flow-injection analysis. Anal. Bioanal. Chem., 372: 822-828.

Tanizawa, H., Y. Ohkawa, Y. Takino, A. Ueno, T. Kageyama and S. Hara, 1992. Studies on natural antioxidants in citrus species. I. Determination of antioxidant activity of citrus fruits. Chem. Pharm. Bull., 40: 1940-1942.

Tial, S., K. Nakamura, T. Cui and H. Kayahara, 2005. High-performance chromatographic determination of phenolic compound in rice. J. Chromatogr. A, 1063: 121-128.

Vinas, P., C. Erroz, J. Hernandez and M. Cordoba, 2000. Determination of phenols in wines by liquid chromatography with photodiode array and fluorescence detection. J. Chromatogr. A, 871: 85-93.