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Determination of gallic acid with rhodanine by reverse flow injection analysis using simplex optimization

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ABSTRACT

A reversed flow injection (rFI) system was designed and constructed for gallic acid determination. Gallic acid was determined based on the formation of chromogen between gallic acid and rhodanine, resulting in a colored product with a λ_{max} at 520 nm. The optimum conditions for determining gallic acid were also investigated. Optimizations of the experimental conditions were carried out based on the so-call univariate method. The conditions obtained were 0.6% (w/v) rhodanine, 70% (v/v) ethanol, 0.9 mol L^{-1} NaOH, 2.0 mL min^{-1} flow rate, 75 μL injection loop and 600 cm mixing tubing length, respectively. Comparative optimizations of the experimental conditions were also carried out by multivariate or simplex optimization method. The conditions obtained were 1.2% (w/v) rhodanine, 70% (v/v) ethanol, 1.2 mol L^{-1} NaOH, flow rate 2.5 mL min^{-1}, 75 μL injection loop and 600 cm mixing tubing length, respectively. It was found that the optimum conditions obtained by the former optimization method were mostly similar to those obtained by the latter method. The linear relationship between peak height and the concentration of gallic acid was obtained over the range of 0.1–35.0 mg L^{-1} with the detection limit 0.081 mg L^{-1}. The relative standard deviations were found to be in the ranges 0.46–1.96% for 1, 10, 30 mg L^{-1} of gallic acid ($n=11$). The method has the advantages of simplicity extremely high selectivity and high precision. The proposed method was successfully applied to the determination of gallic acid in longan samples without interferent effects from other common phenolic compounds that might be present in the longan samples collected in northern Thailand.

1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid) (GA) is an important polyphenolic acid which is widely existed in plants. It has been found to be pharmacologically active as a strong antioxidant, antimutagenic, and anticarcinogenic agent [1–4]. In addition, gallic acid is often used as an indicator of adulteration of fruit juices [5,6] and different alcoholic beverages [7–9]. For instance, cognac and Scotch whisky contain gallic acid [7]; there is a good correlation between the concentration of gallic acid and the age of the beverage.

Several methods have been reported for determination of gallic acid such as electrochemiluminescence [10,11], chemiluminescence [12–14], Liquid chromatography [15,16], and capillary electrophoresis [17]. Determining gallic acid in real samples, according to the analytical/characteristics, has been published in the literature as shown in Table 1. We observed some limitation of the above conventional methods. Those methods require more sophisticated instrument, and cannot be simply adapted for a continuous analysis, high cost of analysis and instrument maintenance, relative long analysis time and risk of toxicity from large volume of toxic/expensive organic solvent for sample pretreatment (e.g., solvent extraction, derivertization prior to HPLC analysis) the conventional mobile phase (methanol or acetonitrile) for separation methods or toxic reagents.

Gallic acid has been determined spectrometrically through complexation with rhodanine [18–20]. The rhodanine assay developed by Thies and Fisher [18] was proved to be extremely, highly selective, no interference from other plant phenolics, to free gallic acid [21]. Only one article based on flow injection spectrophotometric determination of gallic acid using rhodanine has been

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Table 1
The comparative analytical performance between the proposed method and published method.

Analytical characteristic	Chemiluminescence [13]	HPLC [16]	Normal FI [19]	Reverse FI the present work
1. FI-manifold				
Channel	Four-channel	×	Three-channel	Two-channel
Mixing reactor	×	×	×	√
Mixing chamber		×	√ (About 2 mL in volume with a magnetic stirrer)	×
2. Calibration graph				
Regression equation	$\Delta I = 6.9318 + 0.71953\log c$	N.R.	$y = 6.109 + 6032x - 125700x^2$	$y = 0.0599x + 0.0142$
Linear range (mg L^{-1})	1.0×10^{-3}–50	8–140	10–100	0.1–35
Linearity (R^2)	N.R.	0.9996	0.997	0.9989
3. Limit of detection (mg L^{-1})	2.2×10^{-4}	0.31	N.R.	0.081
4. Limit of quantification (mg L^{-1})	N.R.	N.R.	N.R.	0.11
5. Repeatability (%RSD)	1.7	N.R.	> 1.2 ($n = 7$)	0.46–1.96 ($n = 11$)
6. Reproducibility	2.3	N.R.	N.R.	0.87–1.70
7. Accuracy recovery (%)	94.6–103.8	94–96	106	98–102
8. Sample throughput (h^{-1})	120	N.R.	N.R. (t_{base} about 2 min)	35 (t_{base} about 40 s)
9. Reagent consumption (mL h^{-1})	N.R.	N.R.	168	75 μL/sample

*NR = not reported.

published in the literature [19]. Although this common (conventional or normal) flow injection method consumes less reagent than that consumed by batch wise spectrophotometric method, it still consumes rather large volume of reagent (168 mL h^{-1} rhodanine and KOH) [19]. To minimize reagent consumption, reverse flow injection was performed. However, a report involving reversed flow injection (rFI) method for gallic acid determination using rhodanine as the complexing agent has not been yet available in the literature. The normal FI (nFI) technique involves injection of a small volume of standard or sample into a flowing reagent stream [19]. On the contrary, rFI, the reagent is injected into a continuous flowing stream of the sample [22,23]. Unfortunately, nFI, Tygon pump tubes are used to propel reagent (rhodanine) solution stream using alcoholic solution (ethanol) as rhodanine solvent is not recommended in order to guarantee a long lifetime of the Tygon tubing. This drawback was overcome by rFI procedure (reagent was injected). Moreover, the rFI mode has advantages compared to nFI such as minimizing reagent consumption, decreasing sample dispersion, so the analytical sensitivity could also be improved.

In this study, an rFI technique based on the chromogen reaction of the rhodanine assay for gallic acid was developed in order to improve the reproducibility and the sensitivity of the proposed rFI system. Furthermore, this improved method was performed under green chemistry approach including the avoidance of the use of toxic methanol as well as to minimize amount of rhodanine reagent. Comparative optimization of the experimented conditions of the rFI method by the univariate and simplex methods has been performed. The method was tested for gallic acid determination in longan sample extracts.

This work describes a simple, sensitive, selective and inexpensive flow-based (reverse flow injection) method for determination of gallic acid based on the formation of chromogen between gallic acid and rhodanine, resulting in a colored product with λ_{max} at 520 nm. The proposed method was successfully applied to the determination of gallic acid in longan fruit samples.

2. Experimental

2.1. Apparatus

2.1.1. rFI method

The reverse flow injection manifold consisted of a peristaltic pump (Eyela MP3A, Tokyo, Rikakikai Co Ltd., Japan) with the rhodanine reagent solution injected via a six-port injection valve with a 75 μL sample loop (Upchurch Scientific®, model V451). Tygon tubing (C ole-Parmer) with 1.4 mm i.d. was used as flow line for gallic acid standard and/or sample solution, and sodium hydroxide solution, A Y-shaped connector was used for merging the reagent streams. A mixing coil used was made from PTFE tubing (Cole–Parmer), 0.8 mm i.d. for the recommended configuration. The rFI peaks were acquired by using an UV–vis detector (Jenway 6305) coupled with a personal computer (PC).

2.1.2. HPLC method

The HPLC analyses were performed using Varian ProStar 240 Solvent Delivery Module, a binary pump, and a UV detector (Spectra Lab Scientific Inc., CA). Separation was carried out on the VertiSep™ AQS RP-C18 (5 μm, 150 × 4.6 mm i.d.,) column (Vertical Chromatography CO, Ltd.) formic acid and methanol as mobile phase using gradient elution mode. The separated compounds were eluted with gradient system of 0.4% formic acid (solvent A): methanol (solvent B) at a flow rate of 1.0 mL min^{-1}. The injection volume was 10 μL. The gradient system started from 0 min (100% A) to 2 min (95% A), 5 min (70% A), 8 min (100% A) 11 min. The UV detection was set at 270 nm.

2.2. Chemicals, reagents and samples

2.2.1. Chemical and reagents

Most chemicals were of analytical-reagent grade and used without any further purification (unless otherwise specified). De-ionized distilled water was used throughout the whole experiment.

The solution of rhodanine (1.2% w/v) was freshly prepared by dissolving the solid (1.2 g) in ethanol (70 mL) and then diluting with water (30 mL) to give a 1.2 (% w/v) solution, which was stable for over 24 h at room temperature [19]. The stock solution of gallic acid (500 mg L^{-1}) was prepared by dissolving 0.5100 g gallic acid in 1000 mL phosphate buffer (pH 7.4), which was stable for at least one week in a refrigerator. The gallic acid standard solutions were prepared by diluting the stock solution with water. The stock solution of sodium hydroxide (2.0 mol L^{-1}) was obtained by dissolving approximately 8.00 g sodium hydroxide in 100 mL redistilled water and this solution was standardized before use.

2.2.2. Sample

The longan fruits used in this study were collected from Chiang Mai and Lamphum Districts. There cultivars of longan fruits were selected namely Edor, Heaw and Sichompoo. Four samples within the above cultivars of longanal fruits were collected seasonally.