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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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0039-9140/

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.

The samples were (i) Edor No. I (ii) Edor No. II (iii) Heaw and (iv) Sichompoo.

2.3. Procedure

A two-channel rFI manifold with spectrophotometric detection was developed (Fig. 1). The solution of gallic acid and NaOH were pumped continuously with a flow rate at 2.5 mL min^{-1} by a peristaltic pump. A 75 μL reagent solution containing rhodanine was manually injected into flowing carrier stream of standard gallic acid solutions via a six-port injection valve. Then this solution was merged with sodium hydroxide at three-way connector and the product was formed in reaction coil. The maximum absorbance of the product was measured at 520 nm. The plot of rFI signals as peak height against various gallic acid concentrations were used to establish the calibration curve that was employed to evaluate the concentration of gallic acid in the sample solutions.

2.4. Chemometric optimization

A variable-size simplex method was used to optimize the parameters of the rFI system. In order to get a maximum signal to noise ratio in the spectrophotometric determination of gallic acid, three parameters (i.e., mixing coil length, concentration of rhodanine, and NaOH) were changed simultaneously. The sensitivity used for the simplex optimization was based on the basic simplex method [24].

2.5. Sample pretreatment

A selection of ripened longan fruits with approximately the same size was performed. The selected longan fruits were washed and then, all seeds and peels were separated from pulps. After separating, seeds and peels were dried in the oven at 75 °C for 48 h. All samples were separately ground and then stored in the desiccator.

2.6. Sample preparation

Approximately 500 mg each ground and dried longan seed and peel was powder samples accurately weighed and extracted with 5 mL of 75% ethanol for 1 h with the aid of sonication. The sample were centrifuged (2000 g, 3 min, at room temperature), and the supernatants were transferred into 10 mL volumetric flasks. The residues were re-extracted with 4 mL of 75% ethanol. All extracts were combined and filtered through Whatman filter paper (No. 1). All filtrates were evaporated to dryness under vacuum below 40 °C. The residues were dissolved in 10 mL of water and then filtered through a 0.45 μm filter. The samples were diluted 1:99 for longan seed and 1:9 for longan peel using deionised water prior to rFI analysis. The relatively high dilution factor facilitates the elimination of any possible interference and alleviates other matrix effects.

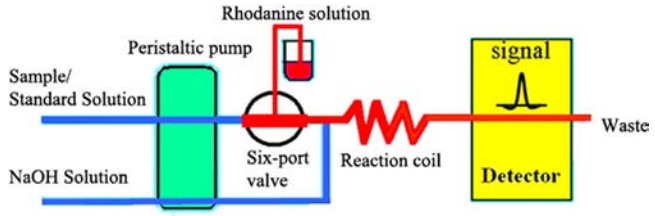

Fig. 1. Schematic description of the rFI system for determination of gallic acid.

2.7. HPLC method

A comparative determination of gallic acid in longan extracts by previously reported procedure [25] was also carried out to evaluate the proposed rFI procedure and verified by Student *t*-test at 95% confident level.

3. Results and discussion

The present work describes a cost effective, simple and greener analytical chemistry method for gallic acid determination. Initially a reverse flow-based system coupled with spectrophotometric detection was developed and tested for gallic acid determination using rhodanine as chromogenic reagent in alcoholic alkaline medium. In the present work ethanolic sodium hydroxide was used instead of methanolic potassium hydroxide to permit greener chemistry purposes.

Generally the dispersion for a FIA system increases with the reaction tubing inner diameter, length and flow rate, and it is inversely proportional to the sample injection volume. The dispersion in nFI is typically in the range 3–10. Increasing dispersion in nFI leads to a decrease in analyte concentration and hence, it also reduce the sensitivity. On the other hand, in the rFI system, the relationship between dispersion and sensitivity are also revered. As soon as the reagent is injected into the flowing sample stream the amounts of sample in the reagent zone increase as the dispersion increases resulting in an enhancement of the sensitivity and well-formed peaks are obtained.

3.1. More detail about the reagent (rhodanine)

Rhodanine and its derivatives had been reported as analytical reagent for spectrophotometric and spot test reagent since 1965 by WI. Stephen and A. Townshen [26]. They exhibited sensitive reactions for Ag(I), Au(III), Cu(II), Pd(II) and Hg(II) by forming water soluble complexes. Rhodanine has been reported for selective spectrophotometric determination of Tannase and gallic acid.

Rhodanine reacts with the vicinal hydroxyl groups of gallic acid to give a red complex with a maximum absorbance at 520 nm, the unreacted rhodanine in basic solution exhibits maximum absorption at 412 nm and no absorption band at wavelengths higher than 450 nm. The gallic acid-rhodanine complex can be conveniently determined at 520 nm with no interference from unreacted rhodanine. It has been reported that reaction with rhodanine provides a specific method for determining gallic acid. This reagent does not react with galloyl esters, elagic acid, catechin, sorghum procyanidine, or chlorogenic acid. Rhodanine reacts with quinones and hydroquinones, but the reaction products give a green color with an absorption at longer wavelenghts (655 nm) than that in the gallic acid-rhodanine complex [27].

Now-a-days, very few published articles are available on spectrophotometric determination of gallic acid based on rhodanine complexation for both batch-wise and flow-based methods [18–20]. Only one paper described flow injection spectrophotometric determination of gallic acid using rhodanine [19]. Based on green chemistry purposes together with the extremely selectivity of rhodanine, no article was published on rFI-spectrophotometric determination of gallic acid which enabled the authors to adopt rhodanine assay method as a basis to develop a novel, highly sensitive rFI method for gallic acid determination in longan sample (the major exported fruit Thailand).

3.2. Reaction mechanism

The possible of gallic acid and rhodanine in ethanol solution might be similar to that reported by Thies and Fischer [18]. Gallic acid in ethanol solution 2 possible resonance forms, as shown in Fig. 2.

In alkaline medium 1 mol of gallic acid reacts with 2 mol of OH^- (as NaOH) resulting in product (II) [18] so that 1/2 mol gallic acid gives rise to 1 mol of product (II). Consider to the reagent rhodanine in alcoholic solution (EtOH) its resonance from is as shown in equation (4) 1 mol of product (II) reacts with 2 mol of rhodanine in ethanolic NaOH result in product (III) as shown in equation 5.

It was shown that 1 mol of gallic acid reacted with 2 mol of rhodanine giving rise to an intense colored complex (Fig. 3).

3.3. Optimization of the reversed flow injection system

The parameters of the rFI system were optimized by two different approaches by a traditional way using the one-variable-at-a-time method (OVATM) and the basic simplex method. In OVATM approach, six variables, i.e., concentrations of rhodanine, solvent of rhodanine, NaOH, mixing coil length, injection loops, and flow rates, were optimized. In basic simplex method approach, three variables, i.e., concentration of rhodanine, NaOH, and mixing coil length, were optimized. Other conditions were those previously optimized by OVATM.

3.3.1. One-variable-at-a-time method (OVATM)

The optimization the OVATM was done by changing one control variable at a time while holding the rest variables at a constant level. The effects of studied parameters on the sensitivity are depicted in Figs. 4–9.

3.3.1.1. Effect of absorption spectra. The absorption spectra of the reagent blank and the colored complex obtained by complexation reaction between gallic acid and rhodanine were measured over the range of 350–700 nm using a spectrophotometer. The absorption maximum of the complex was at 520 nm with a reasonable low blank signal and reproducibility. In order to achieve the greatest sensitivity, measurements were made at 520 nm in further studies.

3.3.1.2. Effect of rhodanine reagent concentration. The influence of rhodanine concentration, used in ethanol solution (70% v/v), on the complex formation was studied in the range of 0.1–1.0 (% w/v). It was found that the highest sensitivity was obtained when the concentration of rhodanine was 0.6%, so this concentration was chosen for further work (Fig. 4).

3.3.1.3. Effect of type and concentration of solvent in rhodanine solution. The effect types of solvent in rhodanine solution were studied by varying the % v/v of methanol and ethanol. It was found that methanol provided the better sensitivity than ethanol, except at 70% ethanol. The sensitivity of ethanol in solution (70% EtOH) was as high as sensitivity that of methanol solution. Due to high toxicity of methanol, 70% ethanol was therefore chosen as the optimum solvent (Fig. 5) for safety purposes.

3.3.1.4. Effect of alkaline solutions. The effect of sodium hydroxide solution concentration was investigated in the range of 0.1–1.0 mol L^{-1}. It was shown that 0.9 mol L^{-1} NaOH was given the highest sensitivity. Thus, 0.9 mol L^{-1} NaOH was chosen as the optimum condition (Fig. 6).

3.3.1.5. Effect of mixing coil length. The effects of various mixing tubing coil lengths for making mixing coil were studied by varying the length from 100 to 900 cm. It was found that the sensitivity

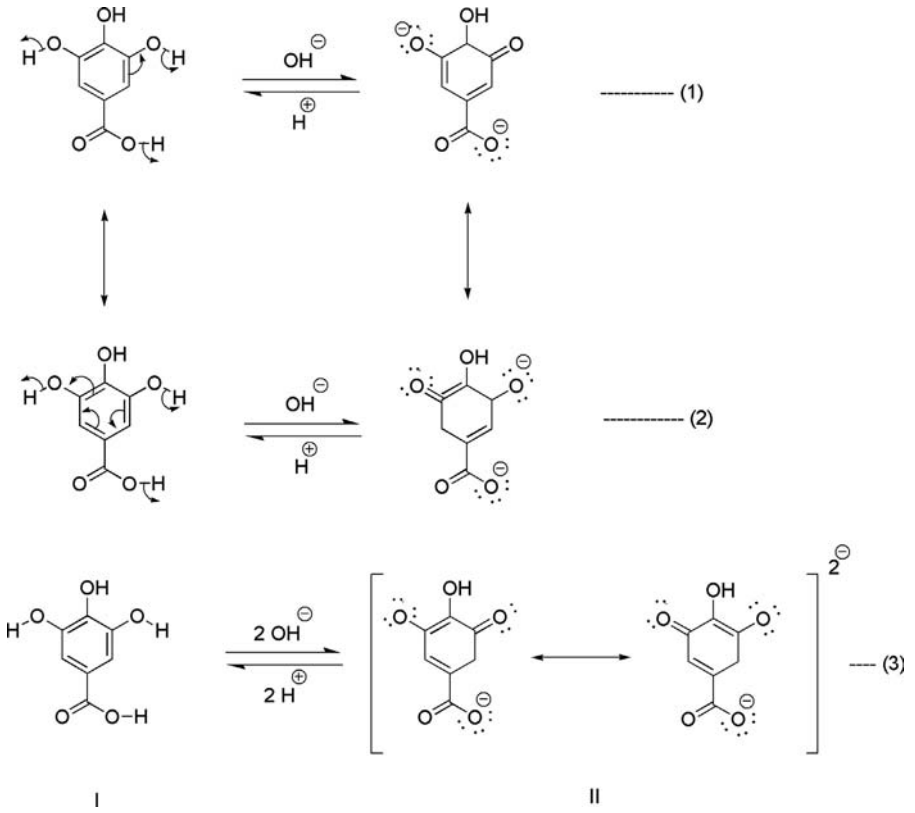

Fig. 2. Resonance forms of gallic acid in ethanolic NaOH solution.

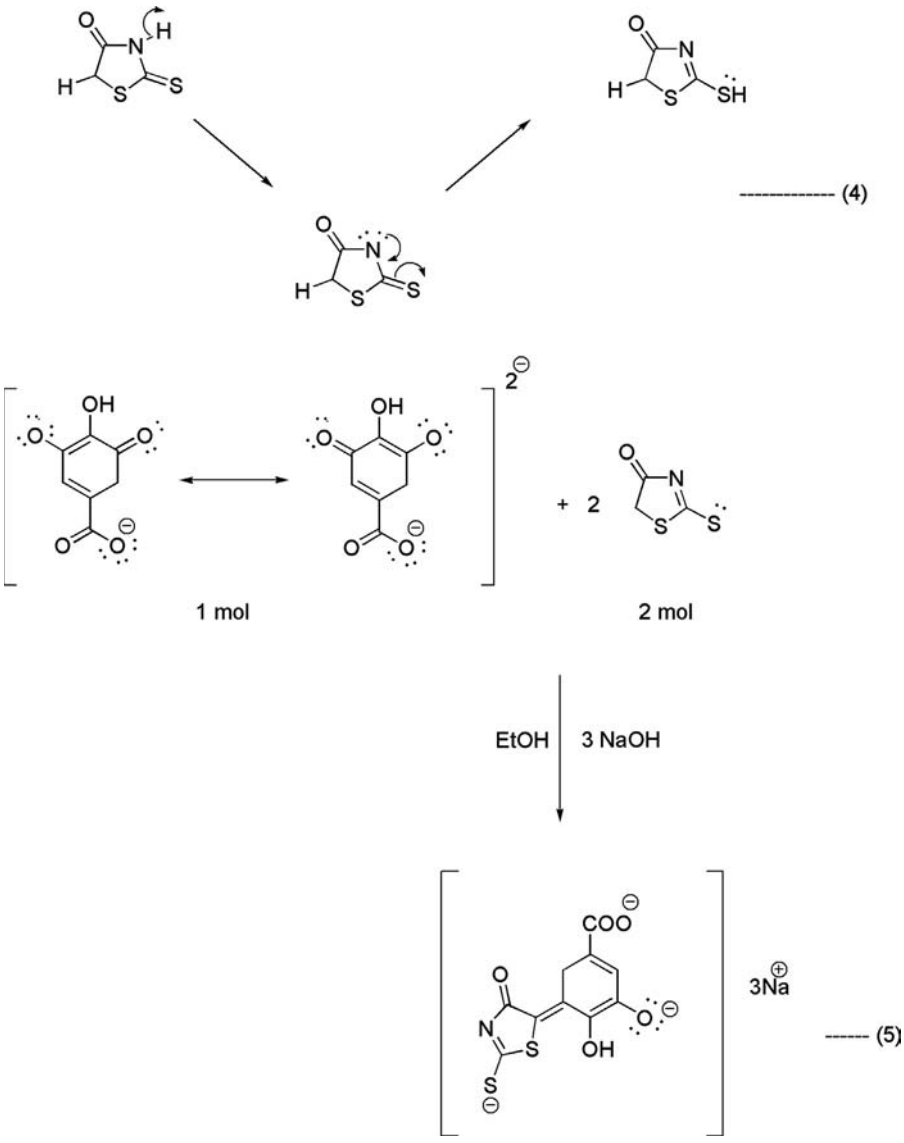

Fig. 3. Reaction mechanism between gallic acid and rhodanine in ethanolic NaOH solution.

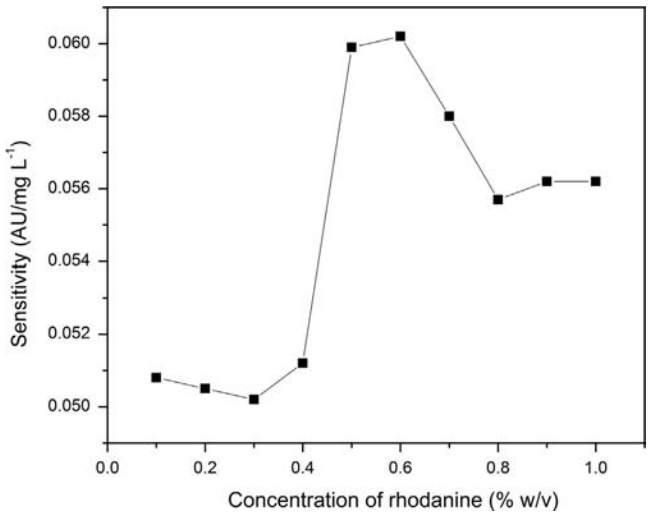

Fig. 4. Effect of rhodanine concentration on the sensitivity of gallic acid-rhodanine complex.

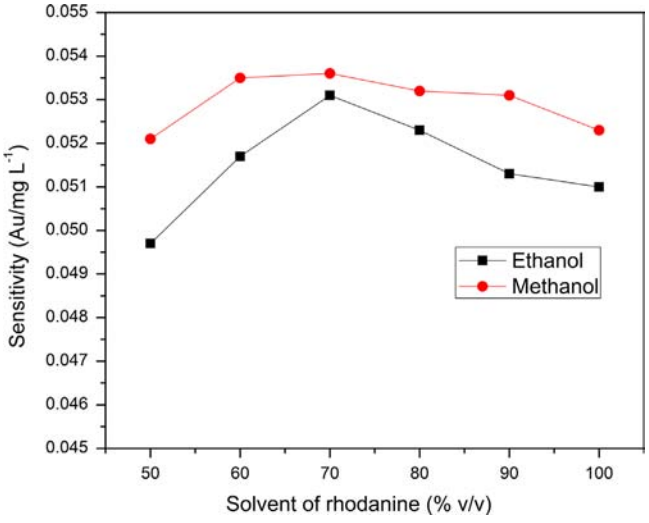

Fig. 5. Effect of solvent of rhodanine concentration on the sensitivity of gallic acid-rhodanine complex.

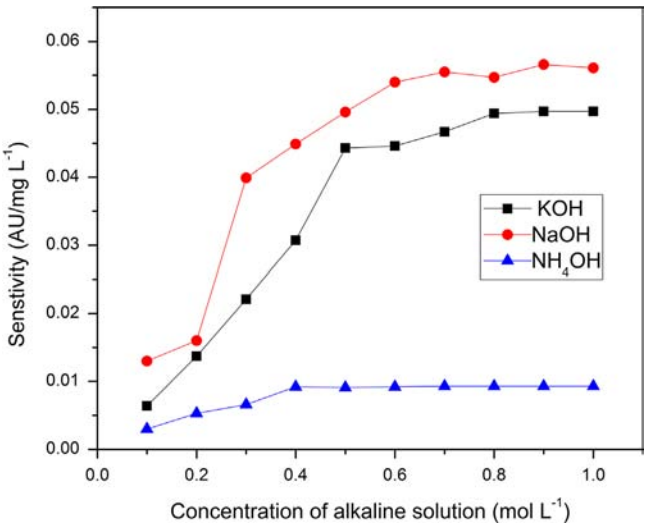

Fig. 6. Effect of NaOH concentration on the sensitivity of gallic acid-rhodanine complex.

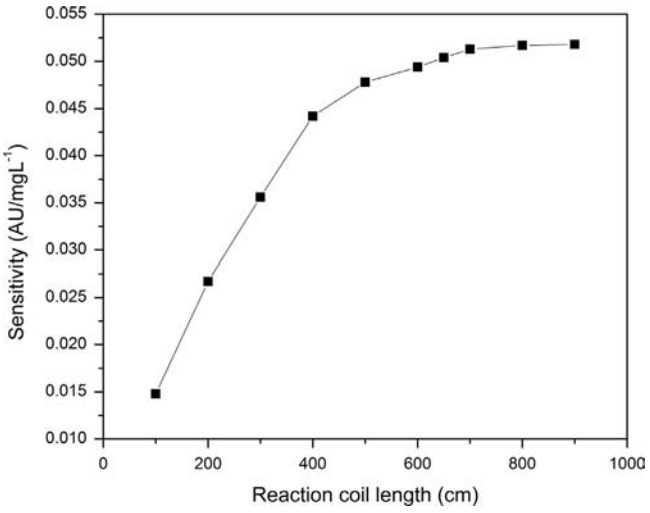

Fig. 7. Effect of mixing coil length on the sensitivity of gallic acid-rhodanine complex.

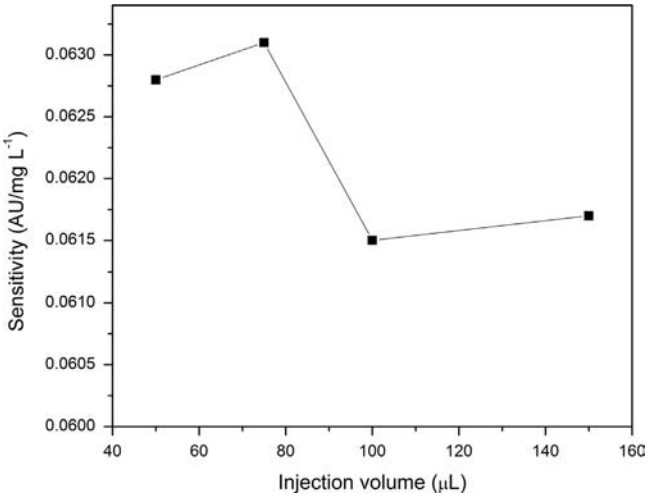

Fig. 8. Effect of injection volume on the sensitivity of gallic acid-rhodanine complex.

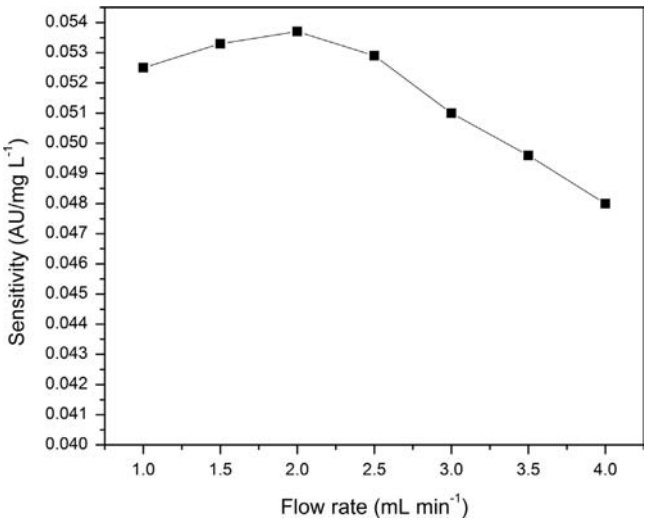

Fig. 9. Effect of flow rate on the sensitivity of gallic acid-rhodanine complex.

increased rapidly up to 600 cm, and then, further increasing to 900 cm the sensitivity was slightly increased. Hence, the 600 cm mixing tubing was chosen as optimum tubing length for making the mixing coil (Fig. 7).

3.3.1.6. Effect of injection loop volume. This study was carried out by injecting the reagent solution using various injection loops with different injection volume ranging between 50 and 150 μL into the flowing sample or standard solution stream containing gallic acid with various concentrations (1, 2, 3 and 4 mg L^{-1}, respectively). The sensitivity of this method does not vary significantly with injection volume while the injection volumes are ranging between 50 and 150 μL. A 75 μL of reagent solution was chosen for further experimental work (Fig. 8).

3.3.1.7. Effect of flow rate. The effect of flow rate of sodium hydroxide and gallic acid standard solution (1, 2, 3, 4 and 5 mg L^{-1}) were investigated over the range of 1.0–4.0 mL min^{-1} for both streams. Maximum sensitivity was obtained at 1.5, 2.0 and 2.5 mL min^{-1}. Thus 2.0 mL min^{-1} was regarded as the optimum flow rate (Fig. 9).

The optimal conditions obtained by univariate method were 2.0 mL min^{-1} flow rate, 75 μL injection volume, and 600 cm mixing coil length. The optimum concentrations of rhodanine, solvent of rhodanine, and NaOH were 0.6%, 70% ethanol, and 0.9 mol L^{-1}, respectively.

3.3.2. Simplex method

Further, to evaluate the results of OVATM, the detection system was also optimized by the simplex method. The optimization by the simplex method is based on an initial design of $k+1$ experiment where k is the number of variables; in this case $k=3$ and the number of initial experiments is 4. After initial experiments, the simplex process continued with evaluation of one new experiment at a given time. The simplex was moved in the direction given by the rules of the variable-sized simplex algorithm, including reflection, expansion and contraction vertices. Changes in the values of variables and signals during the optimization are listed in Table 2. The optimization process was finished after 18 experiments, since there was no further improvement toward the maximization of the signal value. Table 2 shows the variation in the signal value with the experiment number. The conditions for the experiment No. 6 were chosen as optimum, i.e., 1.2% w/v rhodanine, 1.2 mol L^{-1} NaOH, and 600 cm mixing coil length (Fig. 10).

Table 2
The values of variables and signals during optimization by the basic simplex method.

Experiment number	Rhodanine (%w/v)	NaOH (M)	Reaction coil (cm)	Sensitivity (AU/mg L^{-1})
1	0.8	1.0	300	0.0679
2	0.3	0.5	400	0.0606
3	0.4	0.5	500	0.0693
4	0.6	0.7	600	0.0733
5	0.9	1.0	550	0.0771
6	1.2	1.2	600	0.0816
7	0.7	0.6	833	0.0748
8	1.2	1.2	856	0.0814
9	1.5*	1.3	926	**
10	0.8	0.8	681	0.0794
11	1.5*	1.5	591	**
12	0.9	0.8	773	0.0792
13	1.4*	1.3	804	**
14	1.0	1.0	712	0.079
15	1.4*	1.4	627	**
16	1.0	1.0	748	0.0787
17	1.3*	1.3	757	**
18	1.0	1.0	723	0.0781

* Rhodanine is not soluble in 70% ethanol.
** Not detected.

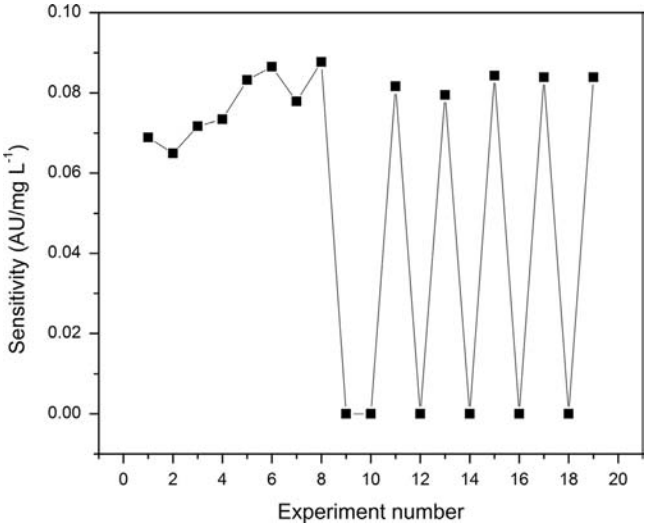

Fig. 10. Process of the basic simplex method optimization.

The optimization by OVATM led mostly to the similar optimal values that were obtained by the basic simplex method but at some parameters the results were different (Table 3).

3.4. Analytical characteristics

3.4.1. Calibration curve linear range and sensitivity

Using the proposed rFI manifold for gallic acid determination under the optimum conditions (Table 3), the linear calibration curve over the range of 0.1–35 mg L^{-1} gallic acid was established which can be expressed by the regression equation $y=0.0599x+0.0142$ ($r^2=0.9989$) where y represents peak height (AU) and x is gallic acid concentration in mg mL^{-1}. Sensitivity defined as slope of calibration graph, which was found to be 0.0599 AU/mg L^{-1} (Fig. 11).

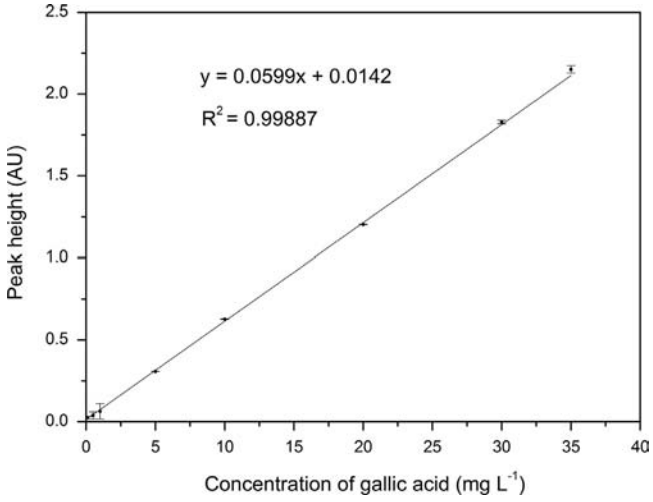

Fig. 11. Calibration graph of GA at concentration of 0.1–35 mg L^{-1}.

3.4.2. Limit of detection (LOD) and limit of quantification (LOQ)

The detection limit was defined as the concentration of analyte that gave the signal that was different from the blank by an amount equal to three times the standard deviation of the blank signal, S_b (3σ, $S_b+3\sigma$ S_b). It was found to be 0.081 mg L^{-1} gallic acid. The quantification limit is defined as the analyte producing a signal that is at least 10 times the standard deviation of the blank signal (10σ, $S_b+10\sigma$ S_b), and was found to be 0.27 mg L^{-1} gallic acid.

3.4.3. Repeatability, reproducibility, accuracy and sample throughput

The accuracy expressed in term of percentage recovery was studied by spiking various concentrations of gallic acid standard solution (5, 10, 20, 30 and 35 mg L^{-1}) into water samples. The percentage recoveries of 5, 10, 20, 30 and 35 mg L^{-1} ($n=5$) of gallic acid were found to be 98, 102, 98, 100 and 101%, respectively. The intraday and inter-day precisions for determining gallic acid (1, 10, 30 mg L^{-1}) were carried out by replicate injections ($n=11$) within the same day were 0.46–1.96%. The procedure was replicated up to 10 day the RSD were over the range of 0.87–1.70%. It was proved that the proposed method provides highly reproducible and accurate results. With respect to the speed (sampling rate sample through put) of the method, it was found that the sample through put was 35 h^{-1} indicating that the proposed rFI method is rapid.

3.4.4. Interferences

Effects of some possible interfering ions on the determination of gallic acid were investigated. Synthetic sample solutions containing 1.0 mg L^{-1} of gallic acid and possible foreign species (cations, anions and certain organic compounds) generally present in plant materials including longans with different concentrations to make the concentration (in mg L^{-1}) of gallic acid to ions from 0.0 up to >1000 were tested. The rFI signal as peak heights were measured under optimal condition (Table 3). All cations, anions and selected organic compounds tested were considered to interfere with the method as soon as changes in peak heights were less than $\pm$ 10% for determining the analyte of interest. No effect was noticed when the mass concentration ratios of the foreign species to the analytes were more than 1000 for Zn^{2+}, Na^+, K^+, NO_3^-, NO_2^-, Cl^-, caffeic acid, catecol, ellagic acid, ferulic acid, 300 for Ca^{2+}, 0.4 for Fe^{3+}, 0.05 for Pyrogallol, respectively. However, the most serious interference from Fe^{3+} and pyrogallol were observed. This problem can be overcome by removed of pyrogallol from

Table 3
The optimum experimental conditions for both optimization methods and the analytical characteristics of simplex method.

Parameters studied	OVATM	Simplex
Wavelength (nm)	520	520
Rhodanine concentration (%w/v)	0.6	1.2
Solvent of rhodanine (ethanol % v/v)	70	70
Sodium hydroxide concentration (mol L^{-1})	0.9	1.2
Mixing coil length (cm)	600	600
Flow rate (mL min^{-1})	2.0	2.5
Injection loop (μL)	75	75
Calibration graph slope (AU/mg L^{-1})	0.0523	0.0599
LOD (mg L^{-1})	0.16	0.081
RSD of 1, 10, 30 mg L^{-1} gallic acid (%) ($n=11$)	2.09–2.93	0.46–1.96

Table 4
Accuracy of proposed rFI method compared with HPLC one for determination of gallic acid.

Samples	Gallic acid found (mg/g)	
	r-FIA	HPLC
Longan seed		
Edor no. 1	1.95	1.90
Edor no. 2	1.89	1.74
Heaw	1.72	1.70
Sichompoo	1.12	1.01
Longan peels		
Edor no. 1	0.26	0.21
Edor no. 2	0.20	0.18
Heaw	0.17	0.20
Sichompoo	0.35	0.37

the plant extract prior to determination of gallic acid by extracting with ether because gallic acid does not dissolve in ether. Alternatively, a minicolumn can be coupled on line to separate pyrogallol and gallic acid prior to determination under suitable conditions [28].

3.4.5. *Analysis of gallic acid in longans*

The rFI system coupled with spectrophotometric detector was employed to quantify gallic acid in longan fruit extract samples (four sample of longan seeds and peels see Section 2.2 (ii)). The concentrations in the sample solutions were determined by reference to the calibration graph prepared under identical experimental conditions. The gallic acid contents in each longan fruit materials was quantified calculating the gallic acid concentration in the original sample solutions which was related to the mass of each solid sample. Gallic acid was parallel measured by HPLC method chosen as reference method. The data summarized in Table 4 prove the good agreements between the proposed and HPLC method (verified by Student's *t*-test).

3.4.6. *Comparison of analytical performance of nFI and the proposed method*

The analytical performance of the proposed method (rFI) was also compared to the nFI. It was found that both method were agreement in analytical performance; accuracy, repeatability. Nevertheless, the proposed method could determine gallic acid in the lower range than nFI. By the comparison, noting that the t_{base} of nFI was higher than rFI, due to the mixing chamber was incorporated into nFI system. In addition, An rFI shows superior minimum reagent consumption, and waste release.

4. Conclusion

The reverse flow injection determination of gallic acid in longan samples based on the formation of chromogen between gallic acid and rhodanine is described. Optimum conditions were investigated by using one-varible-at-a-time method (univariate method) which was agreed well with those obtained by the simplex optimization method (Table 3). To compare results both obtained by optimization procedures it is evident that the simplex method is much faster than the univariate one. In contrast to the time consumed to complete the optimization process, at takes several days for the former optimization approach whereas the latter method takes only one day to complete the optimization procedure. The comparative results of the proposed method with the HPLC method were in good agreement. The advantages of the proposed method are simple, environment friendly (ethanol, as solvent, was used instead methanol), reproducible, sensitive, rapid and cost-effective for gallic acid determination with small volume of reagent consumption and minimum amount of waste production.

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