



Simultaneous Determination of Gallic Acid and Catechins in Banana Peel Extract by Reversed-Phase High Performance Liquid Chromatography

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Abstract

A reversed-phase HPLC method for the simultaneous determination gallic acid, gallo catechin, catechin, epicatechin and epigallocatechin gallate in banana peel extract was developed. The separation was achieved using a reversed-phase (C18) analytical column and an isocratic elution using a mobile phase of acetonitrile-formic acid (15:85 v/v, pH 2.5). The detection wavelength was set at 275 nm. The chromatographic parameters such as peak asymmetry, capacity factor, selectivity factor and resolution factor were determined. Optimization of parameters such as the detection wavelength, the amount of acetonitrile for a mobile phase and the methanol concentration on stability of standard solution was carried out. Under the optimum conditions, the order of elution was gallic acid, gallo catechin, catechin, epicatechin and epigallocatechin gallate, respectively, with the analysis time of 20 min per chromatogram. The validation parameters such as linearity, range, limit of detection, limit of quantitation, selectivity, precision and accuracy, were evaluated. Linear calibration graphs were in range of 0.25–20 mg L⁻¹ for gallic acid and 0.50–30 mg L⁻¹ for gallo catechin, catechin, epicatechin and epigallocatechin gallate, respectively. The proposed HPLC method was simple and rapid, and successfully applied to real samples of banana peel (ripe and green) extracts. Acceptable precisions (%RSD) and recoveries (%Rec) were obtained in the ranges of 0.3–5.9 % and 83±1–128±1 %, respectively, by spiking all samples with a mixed standard solution of five analytes. Banana peel extracts contains many phenolic compounds, especially gallic acid, catechin, epicatechin and epigallocatechin gallate.

Keywords: Gallic acid, Catechins, Isocratic elution, High performance liquid chromatography, Banana peel

Introduction

Banana peel also called banana skin, is the outer covering of the banana pulp. Banana peel is green when fully ripe, its gradually turns yellow and in some cases brown spots are found. The change in pure color is due to degradation of chlorophyll or unmasking of carotenoids (Mohapatra, Mishra, & Sutar, 2010). Banana peel is known as one of banana waste materials which is often ignore and considered as waste for proper utilization as livestock feeds for cattle, poultry, fish, pig, rabbit and several other species. From literatures reviewed, banana peels are rich in nutrients and minerals such as starch (3 %), crude protein (6–9 %), crude fat (3.8–11 %), total dietary fibre (43.2–49.7 %), polyunsaturated fatty acids, particularly linoleic acid and α -linolenic acid, essential amino acids (leucine, valine, phenylalanine, dopamine and threonine), and micronutrients (K, P, Ca, Mg, Fe and Zn). Banana peel is also a good source of lignin (6–12 %), pectin (10–12 %), cellulose (7.6–9.6 %), hemicelluloses (6.4–9.4 %) and galactouronine acid. Various applications of banana peel have been reported to be useful in making banana charcoal, as base material for pectin extraction, as substrate for biogas production, as absorbents for water purification, and in productions of wine, ethanol and banana oil (amyl acetate) (Mohapatra et al., 2010; Pereira & Maraschin, 2015). In



addition for banana peels in conjunction with other substances, it can be created a liniment to relieve the acuteness of the arthritis aches and pains (Anhwange, 2008).

There are many reports in literature determining the antioxidant activities of banana peels in terms of the ABTS scavenging abilities (González-Montelongo, Gloria Lobo, & González, 2010), the DPPH scavenging abilities (González-Montelongo et al., 2010; Sulaiman et al., 2011), total phenolic contents (Fatemeh, Saifullah, Abbas, & Azhar, 2012; González-Montelongo et al., 2010; Sulaiman et al., 2011), and total flavonoid contents (Fatemeh et al., 2012). Moreover, over the past years, several researches focus on natural antioxidant compounds in banana peels, for example, carotenoids as beta-carotene and vitamin A (Pereira & Maraschin, 2015) and phenolic compounds as gallic acid and catechins (del Mar Verde Méndez, Forster, Rodríguez-Delgado, Rodríguez-Rodríguez, & Díaz Romero, 2003; Pereira & Maraschin, 2015; Someya, Yoshiki, & Okubo, 2002) and amine compounds as dopamine (Pereira & Maraschin, 2015). Commonly, phenolic compounds of gallic acid and catechins have strong antioxidant activities associated with their abilities to scavenge free radicals, chelate metals, donate hydrogen, quench singlet oxygen, and break radical chain reactions. The biological properties of these compounds are multifunctional such as anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulant and anti-allergic (Cirillo & Iemma, 2012). Catechins and gallic acid are also the main active substances in other plant sources such as green, oolong and black teas and coffee (Wang, Helliwell, & You, 2000) and flower extract (Samee & Vorarat, 2007).

The contents of gallic acid and catechins in various samples can be determined by several methods, such as electrochemical for green, oolong and black teas and coffee (Kilmartin & Hsu, 2003), capillary electrophoresis for plant (Vaher & Koel, 2003), gas chromatography for banana peel (Waghmare & Kurhade, 2014) and high performance liquid chromatography for banana pulp (del Mar Verde Méndez et al., 2003), flower extracts (Samee & Vorarat, 2007), banana (Someya et al., 2002), rhubarb decoction (Song, Cheng, Tian, & Zhang, 2012) and tea and coffee (Wang et al., 2000), respectively. According to review papers, high performance liquid chromatography (HPLC) with many detectors such as ultraviolet (UV) (Samee & Vorarat, 2007), photodiode array (DAD) (del Mar Verde Méndez et al., 2003; Song et al., 2012; Wang et al., 2000), and mass spectrometer (MS) (Someya et al., 2002) for flower extracts, banana pulp, rhubarb decoction, teas and coffee and banana, respectively, has proved to be the most appropriate method for determination of the structural similarity and diversity of phenolic compounds such as gallic acid, catechin, epicatechin, rutin, ellagic acid and quercetin allowing the analysis with satisfactory sensitivity, selectivity, precision, accuracy and within reasonable time. These previous HPLC methods have some disadvantages such as complex mobile phase (gradient elution) or long analysis time per chromatogram for other samples. These drawbacks can be overcome by a simple isocratic elution and short analysis time per chromatogram for banana peel (Kluai Namwa).

The aim of this study was to optimize, validate and establish a simple and rapid HPLC method for the simultaneous determination of gallic acid (GA) and four catechins (Figure 1) with a separate at constant temperature and at appropriate UV wavelength detection. The four catechins were (+)-catechin (C), (-)-epicatechin (EC), (+)-epigallocatechin gallate (EGCG) and (+)-gallocatechin (GC). The propose method was applied to the analysis these five analytes in crude extracts of both ripe and green banana (Kluai Namwa) peels based on a simplified methanol extraction procedure. The proposed methods offered a short analysis time, acceptable precision and accuracy of the analytical results.

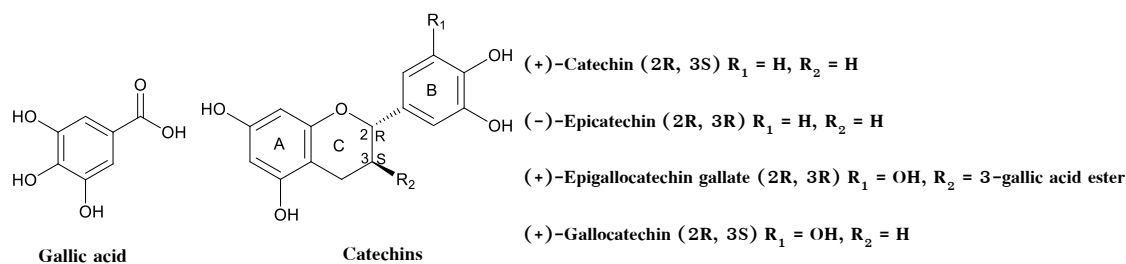


Figure 1 Chemical structures of gallic acid and catechins

Methods and Materials

Chemicals and Reagents

All chemicals were of analytical reagent grade and the solvents were HPLC grade. Ultrapure water was produced using a purification system (Elgastat maxima, Elga, England) with purity of 18.2 M Ω -cm for all aqueous solutions. The standards of gallic acid (98%) and catechin compounds of (+)-catechin (99%), (-)-epicatechin (95%), (+)-epigallocatechin gallate (95%) and (+)-gallocatechin (97%) were obtained from Sigma-Aldrich. The stock standard solutions (1000 mg L⁻¹) were prepared by dissolution of gallic acid in water and catechins in 99.9 %v/v methanol (BDH). These solutions were stored in brown glass bottles and kept at 4 °C for 3 months. Mixed standard solutions were prepared daily by appropriate dilution of the stock solutions with 90 %v/v methanol. Acetonitrile (99.9%, Fisher Scientific) and formic acid (98%, Merck) were used to prepare the HPLC mobile phase. The mobile phase was filtered through a 0.45 μ m nylon membrane filter (Vertical Chromatograph, Thailand) and degassed ultrasonically prior to use.

Banana peel waste samples (Kluai Namwa) of both ripe and green peels were collected from Phitsanulok, Kamphaengphet and Sukhothai provinces and differed in nutrient compositions. This work was focus on the analysis of crude extract samples of the banana ripe and green peels. Therefore, the available crude extract samples used were prepared and received from Kornkanok Ingkaninan and her research group (Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Science, Naresuan University, Phitsanulok, Thailand). All crude extract samples were prepared by extraction of samples with 95 %v/v ethanol, dried, stored in brown glass bottles and kept at 4 °C for further analysis by HPLC method.

Chromatographic equipment and conditions

The HPLC system (Agilent 1100 series system, Agilent, Germany) used consisted of an Agilent G1322A on-line degasser, an Agilent G1311A pump, an Agilent G13290A automatic sample, and an Agilent G1315B photodiode array spectrophotometric detector (DAD). The detector signals, integration of peak areas and determination of retention times were recorded on an Agilent Chemstation software (Agilent, Germany). The column was a VertiSepTM pHendure C18 (particle size 5 μ m, 250 mm x 4.6 mm i.d., Vertical Chromatograph, Thailand). A VertiSepTM pHendure C18 guard column (particle size 5 μ m, 10 mm x 4.6 mm i.d., Vertical Chromatograph, Thailand) was fitted up-stream of the analytical column.

The chromatographic separation was carried out on a VertiSepTM pHendure C18 column. The column temperature was kept constant at 25 °C using an Agilent G1316A column oven. The mobile phase was a mixed solution (15:85 v/v, pH 2.5) of acetonitrile (99.9 %v/v) and formic acid (0.1 %v/v) and must be freshly prepared. All procedures were achieved using isocratic elution with this mobile phase. The flow rate



was set to 1.0 mL min^{-1} . The injection volume was $20 \text{ }\mu\text{L}$ and all standards and sample solutions were injected in triplicate. The optimum wavelength for the determination was 275 nm .

Sample preparation

Aliquots of crude extract samples were weighted (approximately 0.04 g of each sample) and then dissolved with $90 \text{ }\%v/v$ methanol to 1.50 mL in a micro-centrifuged tube. After ultrasonic mixing thoroughly for 10 min , the sample solution was centrifuged for 5 min at 5000 rpm . The supernatant liquid was dried under a stream of nitrogen gas and then dissolved with $200 \text{ }\mu\text{L}$ of $90 \text{ }\%v/v$ methanol into a micro-centrifuged tube. A $50 \text{ }\mu\text{L}$ of this solution was transferred to a micro-centrifuged tube, diluted with $150 \text{ }\mu\text{L}$ of $90 \text{ }\%v/v$ methanol, filtered through a $0.45 \text{ }\mu\text{m}$ nylon membrane filter and kept at $4 \text{ }^\circ\text{C}$ in refrigerator until HPLC analysis.

Validation of the method

The method was validated using the evaluation of variation of retention times and peak areas for analytes by the determination of the following analytical characteristic parameters: linearity, range, limit of detection (LOD), limit of quantitation (LOQ), selectivity, precision and accuracy (Swartz & Krull, 1997). Linearity was measured at seven concentration levels of the mixed standard solutions of each analyte in the investigated ranges from low to high concentration and reported as the variance of the slope of the regression line. Calibration graphs and ranges were constructed by plotting peak area (Y) versus concentration (X) for each analyte using the mixed standard solutions. LOD is the lowest concentration of an analyte that can be detected to assess the detection sensitivity of the analytical method. LOQ is the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy under the stated operational condition of the analytical method. LOD and LOQ were calculated based on 3 and 10, respectively, standard deviations of blank signals (SD_{blank}) and the slope of the calibration graphs at level approaching the LOD and LOQ according to the formulas: $\text{LOD} = 3\text{SD}_{\text{blank}}/\text{slope}$ and $\text{LOQ} = 10\text{SD}_{\text{blank}}/\text{slope}$. Selectivity of the method was evaluated by comparing the chromatograms both of standard and sample solutions at the hand of the peak purity and resolution factors. The precision of the method was expressed as the percentage relative standard deviation (%RSD) by measuring the intra-day precision (repeatability) and the inter-day precision (reproducibility). The accuracy of the method was determined by spiking with two known concentrations of standards into sample solutions and was expressed in terms of the percentage recovery (%Rec).

Results and Discussion

Optimization of chromatographic conditions

In order to verify the applicability of the proposed HPLC method, factors that may affect the chromatographic conditions such as the detection wavelength, the amount of acetonitrile for the mobile phase and the methanol concentration as solvent on stability of standard solutions, were studied thoroughly to optimize the procedure. Referring to the VertiSep™ pHendure C18 technical guide (Application note #686, Vertical Chromatograph, Thailand), the recommended conditions of this reversed-phase C18 column were adapted to find the optimal conditions.

In order to obtain the detection sensitivity and baseline stability, the detection wavelengths of the individual analyte (10 mg L^{-1} of gallic acid and catechins) in $90 \text{ }\%v/v$ methanol, a mobile phase (acetonitrile– 0.1



%v/v formic acid; 15:85 v/v) and a 90 %v/v methanol were studied by continuous scanning in the range of 210–400 nm using the Agilent DAD spectrophotometer. The UV absorption spectra of all chemical solutions are shown in Figure 2. Results showed that the maximum absorption wavelengths (Figure 2(b)) for gallic acid, catechin, epicatechin and epigallocatechin gallate were all at 210 and 275 nm, while gallic acid was at 225 and 255 nm. Moreover, detection at lower wavelengths (between 210–260 nm) gives absorption signals of mobile phase and methanol solutions and gives poorer baseline stability of each analyte (Figure 2(a)). By compromising the acceptable detection sensitivity, base line stability and no peak signals of solvents and impurities, the detection wavelength was selected and kept constant at 275 nm with great facility to use in this work.

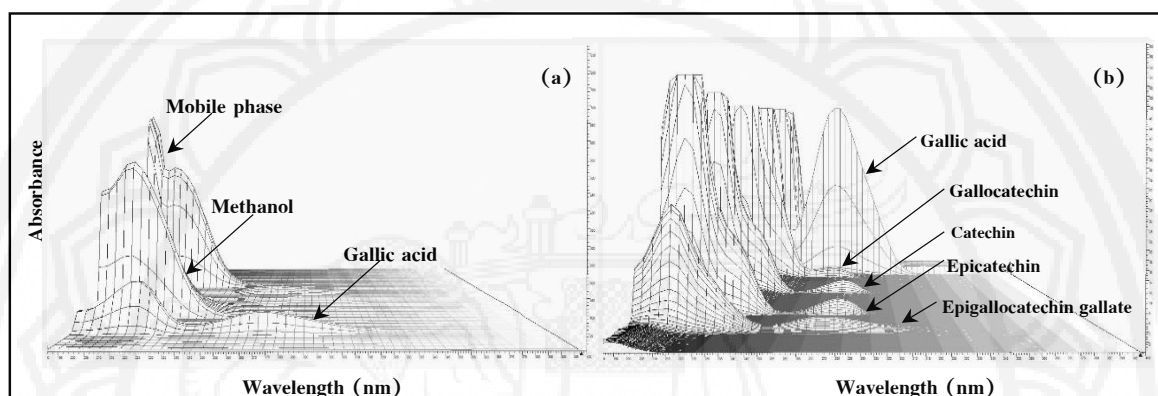


Figure 2 Absorption spectra of a mobile phase (acetonitrile – 0.1 %v/v formic acid; 15:85 v/v), methanol (90 %v/v), gallic acid (10 mg L^{-1}) and catechins (10 mg L^{-1} of gallic acid, catechin, epicatechin, and epigallocatechin gallate)

Generally, for a reversed-phase HPLC, the retention time would decrease when the amount of non-polar mobile phase increases. Thus, the effect of the amount of acetonitrile in various mobile phase compositions of acetonitrile and 0.1 %v/v formic acid (10:90, 15:85 and 20:80 v/v) was studied to obtain an appropriate retention time and analysis time per chromatogram with an acceptable resolution factor and to avoid peak tailing of compounds. It was cleared that the higher amount of acetonitrile resulted in shorter retention times of all analytes, especially effective to catechin, epicatechin and epigallocatechin gallate, and also gave shorter analysis time per chromatogram as shown in Figure 3(a). With the acetonitrile–formic acid at 10:90 v/v, all analytes were well separated with reducing peak tailing and presented an analysis time per chromatogram at 45 min. Under the usage of the acetonitrile–formic acid at 20:80 v/v, peaks of gallic acid and gallic acid were not well separated with a resolution factor < 1.5 . Based on these data, a satisfactory analysis time (20 min per chromatogram), a good resolution and an acceptable peak tailing, the mobile phase of acetonitrile–formic acid at 15:85 v/v was chosen for the further experiments.

Nevertheless, many HPLC conditions should be investigated such as the pH of mobile phase, flow rate and column temperature. These conditions effects in many reasons including (1) the tolerance of a VertiSep™ pHendure C18 column to acid is lowest at pH 1, (2) the five analytes (the pK_a in the range of 4.40–8.72) should be in undissociated form during the analysis, and (3) all analytes are good separation and kept the precise retention times. For convenience, in this work, the pH of mobile phase, flow rate and column temperature were not investigated. Therefore, from the HPLC column technical guide, the pH of mobile phase,

flow rate and column temperature were used and kept constant at 2.5, 1.0 mL min⁻¹ and 25 °C, respectively, in subsequent analyses.

According to review papers (Arts & Hollman, 1998; del Mar Verde Méndez et al., 2003), methanol could be used as a solvent to protect the decomposition of the five analyte compounds. Thus, in this study, methanol was selected as a solvent for the preparation of sample and standard solutions prior to HPLC analysis. Because, on the one hand, the automatic sample system was done for the whole analysis, mostly more than 24 hours during routine analysis. In order to obtain the stabilization in the presence of time, the detection sensitivity and peak symmetry of all analytes, the effect of methanol concentration was studied at 50 and 90 %v/v within the difference injection times between 0–72 hours. It was found that gallic acid, gallo catechin, catechin and epicatechin were stable in both methanol concentrations during the time period while epigallocatechin gallate was rapid decompositions at 50 %v/v methanol after 12 hours (Figure 3(b)), respectively. Moreover, the detection sensitivity (peak area) of all analytes at 90 %v/v methanol was less than in 50 %v/v methanol and the tailing peak of catechin, epicatechin and epigallocatechin gallate in 90 %v/v methanol was slightly occurred. Nevertheless, in this study, the 90 %v/v methanol was selected for the sample and standard preparations which was no decomposition of all analytes, acceptable detection sensitivity and acceptable peak tailing within the resolution > 1.5 (summarized in Table 1).

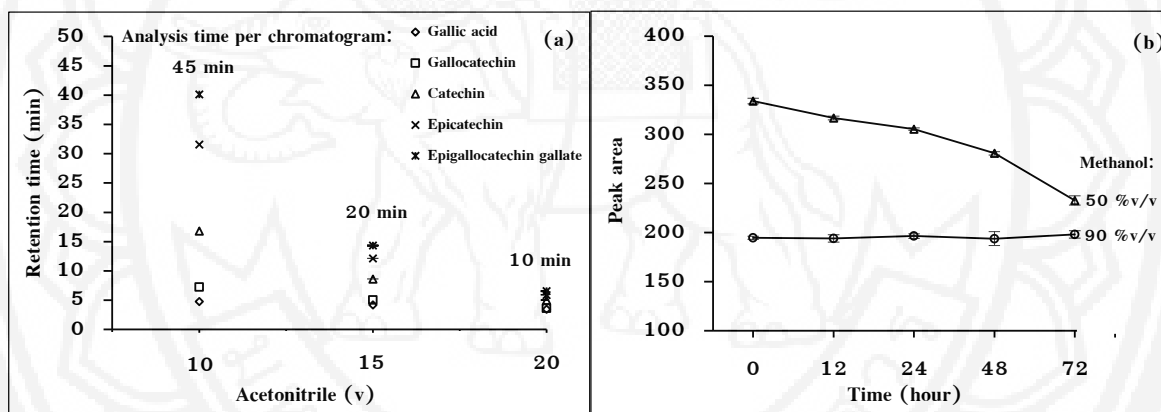


Figure 3 (a) Effect of the amount of acetonitrile (volume (v); mL) in 0.1 %v/v formic acid for the mobile phase (v/v), and (b) effect of methanol concentration on stability of epigallocatechin gallate within the difference injection times between 0–72 hours

Under the conditions used as described above, the typical chromatogram of a mixed standard solution of the five analytes was illustrated in Figure 4 with an analysis time of 20 min. The order of elution was gallic acid, gallo catechin, catechin, epicatechin and epigallocatechin gallate with the retention times (t_R) of 3.86 ± 0.06 , 4.65 ± 0.07 , 7.95 ± 0.12 , 11.19 ± 0.14 and 13.24 ± 0.17 min, respectively. In addition, various chromatographic parameters were calculated to perform the chromatographic resolution and the efficiency of the proposed HPLC system for the simultaneous determination of five analytes. These parameters were such as peak asymmetry (A), capacity factor (k'), selectivity factor (α) and resolution factor (R) were obtained in Table 1. It can be seen that the peak asymmetry factors resulted lower than the acceptable value ($1 < A < 1.2$) for gallic acid and gallo catechin while catechin, epicatechin and epigallocatechin gallate were greater than 1.2 and not more than 2 (unacceptable value). The capacity factors (resulted and kept the acceptable value between 1 and 10; $1 < k'$



< 10), selectivity factors (resulted greater than acceptable value; $\alpha > 1$) and resolution factors (gained greater than 1.5 between two peaks; $R > 1.5$) were found to be satisfactory for the HPLC system.

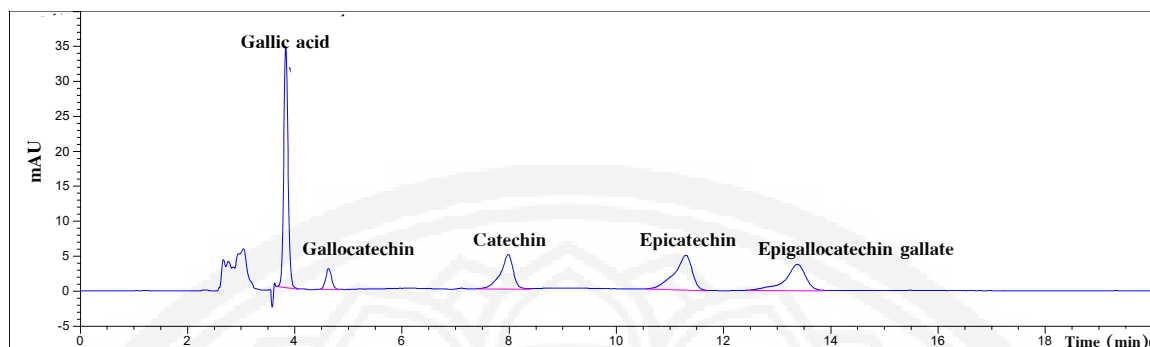


Figure 4 Chromatogram of a mixed standard solution (5 mg L^{-1} of gallic acid and 10 mg L^{-1} of gallocatechin, catechin, epicatechin and epigallocatechin gallate, respectively)

Table 1 Chromatographic parameters obtained in the HPLC system of gallic acid and catechins determination

Analyte	Peak asymmetry (A)	Capacity factor (k')	Selectivity factor (α)	Resolution factor (R)
Gallic acid	1.1	1.0	–	–
Gallocatechin	1.1	1.0	–	–
Catechin	1.5	2.1	–	–
Epicatechin	1.5	3.3	–	–
Epigallocatechin gallate	1.4	4.1	–	–
Gallic acid–Gallocatechin	–	–	1.6	5.4
Gallocatechin–Catechin	–	–	2.6	14.8
Catechin–Epicatechin	–	–	1.6	12.4
Epicatechin–Epigallocatechin gallate	–	–	1.2	4.9

Method validation

The method validation of the HPLC assay was established through the study. From the results, the calibration data and the LOD and LOQ values of five analytes are concluded in Table 2. Linearity of each analyte was obtained the calibration graphs over the range of $0.25\text{--}20 \text{ mg L}^{-1}$ for gallic acid and $0.50\text{--}30 \text{ mg L}^{-1}$ for gallocatechin, catechin, epicatechin and epigallocatechin gallate, respectively. The linear correlation coefficients (r^2) were all above 0.999. Precisions (RSD) obtained from triplicate injections of the standard solutions containing each analyte was less than 4.1 %.

With the selected conditions used, all five analytes were well separated on the VertiSep™ pHendure C18 column and the optimal mobile phase with an acceptable peak asymmetry and resolution factors, but when validating of the method by comparing the chromatograms both of standard and sample solutions, we found it suitable only for the determination of four analytes (gallic acid, catechin, epicatechin and epigallocatechin gallate) in samples of both ripe and green banana peel extracts. Possible interference of an interfering peak at retention time of $4.99 \pm 0.05 \text{ min}$ was overlapped on the gallocatechin peak. The resolution factor (R) between this interfering peak and gallocatechin was less than 0.7. Thus, this proposed HPLC method was not selective for the determination of gallocatechin.



The intra-day of the method were determined by nine repeated analyses of the mixed standard solutions of five analytes at two different concentration levels (4 and 8 mg L⁻¹ of gallic acid and 8 and 15 mg L⁻¹ of gallo catechin, catechin, epicatechin and epigallocatechin gallate). The inter-day precisions of the method were determined day by day during 3 days with the same concentrations as intra-day assay. The proposed method was found to be precise within 0.9–1.9 %RSD for intra-day and 1.4–3.0 %RSD for inter-day (summarized in Table 3).

Table 2 Calibration data of five analytes determination by the proposed HPLC method.

Analyte	Linear range (mg L ⁻¹)	Linear equation (y=ax+b)	r ²	% RSD ^a	LOD ^b (mg L ⁻¹)	LOQ ^c (mg L ⁻¹)
Gallic acid	0.25–20	y = 41.57x-5.20	0.9998	0.3–1.5	0.01	0.04
Galocatechin	0.50–30	y = 2.65x-1.22	0.9991	0.3–3.0	0.07	0.22
Catechin	0.50–30	y = 8.35x+1.37	0.9993	0.3–2.0	0.10	0.32
Epicatechin	0.50–30	y = 11.74x-2.10	0.9991	0.8–4.1	0.01	0.04
Epigallocatechin gallate	0.50–30	y = 13.72x-3.53	0.9991	0.1–4.1	0.02	0.07

^a Percentage relative standard deviation of different concentrations of each analyte in the calibration range

Table 3 Precision of the proposed HPLC method for the determination of five analytes

Analyte	Concentration (mg L ⁻¹)	Intra-day (n=9)		Inter-day (n=9)	
		Peak area	RSD (%)	Peak area	RSD (%)
Gallic acid	4	165.02±1.66	1.0	163.80±3.08	1.9
	8	355.01±0.18	1.0	357.79±5.03	1.4
Galocatechin	8	17.49±0.21	1.2	17.71±0.31	1.7
	15	36.53±0.70	1.9	36.76±0.87	2.4
Catechin	8	75.16±1.07	1.4	76.34±2.20	2.9
	15	126.76±2.04	1.6	126.22±2.83	2.2
Epicatechin	8	95.38±1.86	1.9	94.49±2.72	2.9
	15	167.99±3.13	1.9	171.32±5.09	3.0
Epigallocatechin gallate	8	106.33±1.16	1.1	105.54±1.43	1.4
	15	218.07±1.90	0.9	217.22±3.66	1.7

Application to real samples of banana peel extracts

According to the HPLC method as described above, the gallic acid and catechins in real samples of banana peel extracts of both ripe and green peels, were analyzed. Eight real samples of green (P1–P4) and ripe (PR5–PR8) peel extracts were diluted using an appropriate dilution factor and analyzed in triplicate. Typical chromatograms are shown in Figure 5. It can be seen that the peaks of gallic acid, catechin, epicatechin and epigallocatechin gallate were well separated from the interfering peaks in all samples. No detectable amounts of gallo catechin were observed in all samples because of low concentrations and an overlap with other interference species. The results obtained are summarized in Table 4. The results indicated that gallic acid and epicatechin were found in all ripe and green peel samples with low contents in the range of 3±0.01–18±0.04 mg L⁻¹ and 25±0.1–183±0.3 mg L⁻¹, respectively. Catechin and epigallocatechin gallate were found with



low contents in some samples. For the method validation, spiking diluted test samples with a mixed standard solution of five analytes at two different concentration levels (4 and 8 mg L⁻¹ of gallic acid, and 8 and 15 mg L⁻¹ of gallo catechin, catechin, epicatechin and epigallocatechin gallate, respectively), gave recoveries and precision (RSD) in the ranges of 83±1–128±1 % and 0.3–5.9 %, respectively.

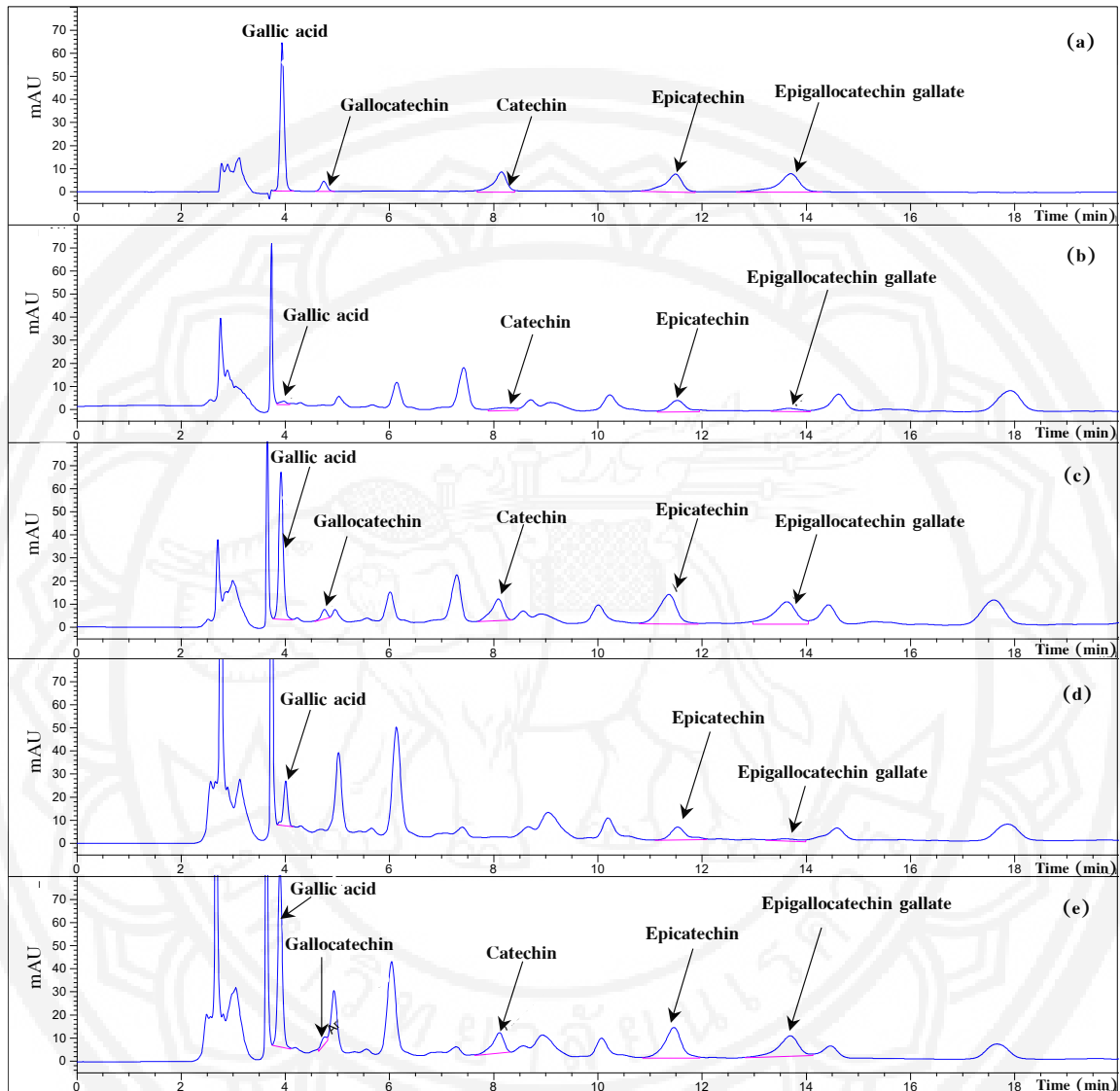


Figure 5 Typical chromatograms of: (a) a mixed standard solution (gallic acid (8 mg L⁻¹), gallo catechin (15 mg L⁻¹), catechin (15 mg L⁻¹), epicatechin (15 mg L⁻¹) and epigallocatechin gallate (15 mg L⁻¹)), (b) a banana green peel extract, (c) a banana green peel extract + a mixed standard solution, and (d) a banana ripe peel extract, (e) a banana ripe peel extract + a mixed standard solution



Table 4 Contents (mg L^{-1}) of four analytes in real samples of banana peel extracts of both green and ripe peels, as determined by the proposed reversed-phase HPLC method were obtained by spiking with the mixed standard solutions containing 4 and 8 mg L^{-1} of gallic acid and 8 and 15 mg L^{-1} of catechin, epicatechin and epigallocatechin gallate, respectively

Sample No.	Banana peel	Added (mg L^{-1})		Concentration found, %relative standard deviation and %recovery (n=3)					
		GA	C	Gallic acid			Catechin		
				mg L^{-1} ^c	%RSD	%Rec	mg L^{-1}	%RSD	%Rec
1		0	0	1.8±0.04 ^d (18±0.04) ^e	2.2	-	2.3±0.1 (23±0.1)	4.3	-
2	P1 ^a	4	8	5.6±0.04	0.7	95±1	10.2±0.1	1.0	99±2
3		8	15	9.7±0.4	4.1	99±5	16.8±0.2	1.2	97±1
4		0	0	0.3±0.01 (6±0.01)	3.3	-	1.7±0.1 (34±0.1)	5.9	-
5	P2 ^a	4	8	4.4±0.1	2.3	103±4	10.1±0.2	2.0	105±2
6		8	15	9.2±0.1	1.1	111±2	18.5±0.3	1.6	112±2
7		0	0	0.7±0.02 (3±0.02)	2.8	-	1.8±0.01 (7±0.01)	0.5	-
8	P3 ^a	4	8	5.0±0.04	0.8	108±1	10.3±0.1	1.0	106±1
9		8	15	9.3±0.03	0.3	108±1	17.9±0.1	0.6	107±1
10		0	0	0.6±0.01 (3±0.01)	1.7	-	4.7±0.2 (19±0.2)	4.3	-
11	P4 ^a	4	8	4.6±0.1	2.2	100±2	13.7±0.2	1.5	112±4
12		8	15	9.5±0.05	0.5	111±1	21.7±0.7	3.2	113±5
13		0	0	2.2±0.1 (11±0.1)	4.5	-	N.D. ^f	-	-
14	PR5 ^b	4	8	6.5±0.1	1.5	107±3	8.5±0.4	4.7	106±5
15		8	15	11.1±0.1	0.9	111±2	17.4±0.5	2.8	116±4
16		0	0	1.7±0.1 (13±0.1)	5.9	-	1.8±0.1 (14±0.1)	5.5	-
17	PR6 ^b	4	8	5.7±0.05	0.9	100±2	9.7±0.2	2.1	99±4
18		8	15	9.6±0.1	1.0	99±2	17.7±0.2	1.1	106±2
19		0	0	2.4±0.02 (10±0.02)	0.8	-	N.D.	-	-
20	PR7 ^b	4	8	6.9±0.1	1.4	113±2	6.6±0.1	1.5	83±1
21		8	15	10.9±0.1	0.9	106±2	13.6±0.5	3.7	91±3
22		0	0	2.9±0.1 (12±0.1)	3.4	-	2.1±0.02 (8±0.02)	0.9	-
23	PR8 ^b	4	8	6.7±0.1	1.5	94±3	9.9±0.1	1.0	98±2
24		8	15	10.8±0.1	0.9	99±2	17.2±0.2	1.2	101±2

Sample No.	Banana peel	Added (mg L^{-1})		Concentration found, %relative standard deviation and %recovery (n=3)					
		EC	EGCG	Epicatechin			Epigallocatechin gallate		
				mg L^{-1} ^c	%RSD	%Rec	mg L^{-1}	%RSD	%Rec
1		0	0	12.4±0.2 ^d (124±0.2) ^e	1.6	-	N.D. ^f	-	-
2	P1 ^a	8	8	20.6±0.1	0.5	103±2	8.0±0.2	2.5	100±3
3		15	15	27.4±0.6	2.2	100±4	15.3±0.4	2.6	102±3
4		0	0	9.1±0.3 (183±0.3)	3.3	-	2.3±0.1 (47±0.01)	4.3	-
5	P2 ^a	8	8	18.4±0.1	0.5	116±4	10.2±0.4	3.9	99±7
6		15	15	28.3±0.2	0.7	128±1	18.8±0.5	2.7	110±5
7		0	0	8.0±0.1 (32±0.1)	1.3	-	N.D.	-	-
8	P3 ^a	8	8	15.9±0.3	1.9	99±3	8.2±0.1	1.2	103±1
9		15	15	22.9±0.3	1.3	99±2	15.3±0.1	0.7	102±1
10		0	0	6.1±0.1 (25±0.1)	1.6	-	N.D.	-	-
11	P4 ^a	8	8	14.0±0.1	0.7	99±1	8.4±0.1	1.2	105±2
12		15	15	21.0±0.6	2.9	99±4	16.0±0.1	0.6	107±1
13		0	0	7.0±0.1 (35±0.1)	1.4	-	1.0±0.03 (5±0.03)	3.0	-
14	PR5 ^b	8	8	15.7±0.4	2.5	109±5	8.4±0.3	3.5	93±4
15		15	15	24.2±0.3	1.2	115±3	16.7±0.6	3.6	105±5
16		0	0	10.3±0.4 (82±0.4)	3.8	-	N.D.	-	-
17	PR6 ^b	8	8	18.5±0.2	1.1	103±6	9.4±0.2	2.1	118±2
18		15	15	25.9±0.6	2.3	104±4	16.8±0.3	1.8	112±2
19		0	0	8.3±0.3 (33±0.3)	3.6	-	N.D.	-	-
20	PR7 ^b	8	8	16.6±0.1	0.6	104±3	8.7±0.3	3.4	109±7
21		15	15	24.0±0.6	2.5	105±4	15.4±0.4	2.5	103±5
22		0	0	7.5±0.2 (30±0.2)	2.7	-	N.D.	-	-
23	PR8 ^b	8	8	16.0±0.3	1.9	106±5	8.7±0.2	2.2	109±2
24		15	15	22.7±0.3	1.3	101±3	15.0±0.1	0.7	100±1

^a P1 to P4 – referred to green peel samples of banana peel extracts, ^b PR5 to PR8 – referred to ripe peel samples of banana peel extracts, ^c Average value ± standard deviation of triplicate results ($\bar{X} \pm \text{SD}$), ^d Concentrations were found by comparing the calibration graph of each analyte, ^e Actual concentrations were obtained by calculating dilution factor of each sample, ^f N.D. – not detected (or < LOD).



Conclusion

In this study, a reversed-phase HPLC method using a simple isocratic elution (acetonitrile–formic acid at 15:85 v/v) and a wavelength detection at 275 nm have been developed and validated for the simultaneous determination of gallic acid, gallocatechin, catechin, epicatechin and epigallocatechin gallate. The present method is simple, rapid (20 min per chromatogram) and does not require any complicated sample preparation. Moreover, the proposed method offers good linearity, sensitivity, precision and acceptable accuracy and can be suitable for routine analysis without advanced facilities. This method was successfully applied to real samples of banana peel extract for the analysis of only four analytes such as gallic acid, catechin, epicatechin and epigallocatechin gallate. The main disadvantage of the proposed method is the fact that lower contents of gallocatechin in samples could be not determined and that gallocatechin has the close interfering peaks. In addition, the results present that natural antioxidants (gallic acid, catechin, epicatechin and epigallocatechin gallate) can be found in banana peel extracts in both ripe and green peels and these peels should receive more attention for other applications.

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