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Determination of Rutin in Buckwheat Tea and *Fagopyrum tataricum* Seeds by High Performance Liquid Chromatography and Capillary Electrophoresis

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ABSTRACT

Simple, fast and very sensitive high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) methods for the determination of rutin were developed. The separation of rutin by HPLC was performed using a Chromolith RP 18 column with detection wavelength at 356 nm. Composition of mobile phase was methanol : water (50 : 50, v/v) in 10 mM acetate buffer at pH 4.1. The limit of detection (at a signal-to-noise ratio of 3) was 23.91 ng/mL. Relative standard deviations of retention time, peak area and peak height (n = 5) for rutin were 0.199, 0.832 and 0.522%, respectively. For CE method, rutin was separated on a bare fused silica column. Borate buffer at pH 9.4 was used as the background electrolyte and detected wavelength was 208 nm. The limit of detection (at a signal-to-noise ratio of 3) was 55.67 ng/mL by using a 5 s injection time and +20 kV power supply. Relative standard deviations of retention time, peak area and peak height (n = 5) were 0.377, 0.923 and 2.446%, respectively. Both methods were applied to determine rutin in buckwheat tea and *Fagopyrum tataricum* seeds. Sample matrix in buckwheat tea was removed by using Sep-Pak C18 prior injection to HPLC and CE. The results for rutin determination obtained by the HPLC method agreed well with those from CE method.

Key words: rutin, buckwheat tea, *Fagopyrum tataricum* seeds, high performance liquid chromatography, capillary electrophoresis

INTRODUCTION

Rutin is a flavonol glycoside and its main function in the body is to strengthen arteries and veins[(1,2)] and harden bones and teeth[(3)]. It is found in many plants, fruits and vegetables[(4-8)]. The richest source is in buckwheat, especially in tartary buckwheat (*Fagopyrum Tataricum*). A previous paper[(9)] reported tartary buckwheat seeds contained higher concentration of rutin than common buckwheat seeds and rutin content in seeds depends on variety and growing conditions. Buckwheat is recognized as a health food in several countries such as Japan, China, Taiwan and Korea, often add buckwheat into noodles and soft drinks. Various names are used for rutin such as rutoside, quercetin-3-rutinoside and sophorin. IUPAC name is 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl] oxymethyl]oxan-2-yl]oxychromen-4-one and its structure is displayed in Figure 1. Studies have demonstrated that rutin helps to stop venous edema, which is an early sign of chronic venous disease of the leg. Animal studies have shown that

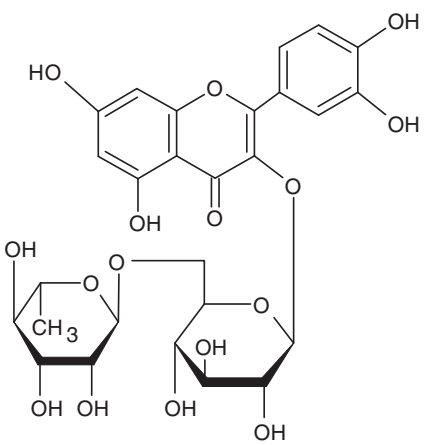

Figure 1. Structure of rutin.

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rutin has preventive and healing effects. There are indications that rutin can inhibit some cancerous and precancerous conditions. In addition, rutin can inhibit platelet aggregation which making the blood thinner and improving the circulation. It also inhibits aldose reductase activity, therefore sorbitol which produces from glucose by using aldose reductase enzyme is reduced, leading to prevent or delay of diabetes[10]. Rutin has strong antioxidant properties. It may help prevent the oxidation of vitamin C and also reduces the cytotoxicity of oxidized LDL cholesterol and lower the risk of heart disease[11]. With several advantageous properties of rutin, it was added into various foods and soft drinks. For quality control, the development of fast and high sensitivity methods for the determination of rutin in samples without interference is required.

High performance liquid chromatography[2,12-22] and capillary electrophoresis[4,6-8,23-27] were the commonly used methods for the determination of rutin by using spectrophotometric and electrochemical detectors. The advantage of these methods was for multiple compounds in samples. Therefore, the methods were time-consuming (Table 1). A small number of papers were directed towards the detection of rutin in samples. For example, the determination of rutin in oral dosage forms based on an isocratic RP-HPLC was developed on bonded phase column with the limit of detection of 2600 ng/mL at retention time of 2.3 min[21]. In our knowledge, no previous HPLC was developed for the determination of rutin on monolithic RP 18 column. In addition, the comparison of HPLC and CE for determination of rutin in dried herbal drug was optimized and limit of detections were 700 ng/mL and 900 ng/mL, respectively[27]. Therefore the aim of this research was to develop simple, fast and more sensitive HPLC and CE methods for the determination of rutin. The separation was performed on monolithic RP 18 column and bared fuse silica column by HPLC and CE, respectively. Then both HPLC and CE methods were applied to determine rutin in buckwheat tea and *Fagopyrum tataricum* seeds.

MATERIALS AND METHODS

I. *Chemicals and Samples*

A stock solution of rutin (Wako Pure Chemical Industries, Osaka, Japan) was prepared in methanol-water (40 : 60, v/v) at a concentration of 100 mg/L. For linearity studies, rutin was diluted as appropriate with deionized water. Chemicals were of analytical grade. Sodium tetraborate (Aldrich, Steinheim, Germany), HPLC methanol (Merck, Darmstadt, Germany), boric acid (Aldrich, Steinheim, Germany) and acetic acid (BDH, Poole, UK) were used. Due to high concentration of rutin in tartary buckwheat and interested in nutrition value added in soft drink, therefore, samples of *Fagopyrum tataricum* seeds and buckwheat tea obtained from Japan were selected for the determination of rutin in this research.

II. *Instrumentation and Conditions*

(I) *HPLC*

Separations were performed using a Waters (Milford, MA, USA) Model 600E gradient pump, a Rheodyne (Cotani, CA, USA, model 7125) stainless steel injector (5 µL loop), a UV-VIS detector (Jasco, Tokyo, Japan) operated at 356 nm. A Chromolith RP-18 endcapped column (4.6 mm i.d., 100 mm, Merck, Darmstadt, Germany) was used as the analytical column and was fitted with a Chromolith RP-18 (4.6 mm i.d., 5 mm, Merck, Darmstadt, Germany) guard column. Mobile phase was methanol-water (50 : 50%, v/v) containing 10 mM acetate buffer at pH 4.1. The flow rate of the mobile phase was 1.5 mL/min and the column temperature was kept at 30°C. Sep-Pak C18 cartridges (360 mg, 55-105 µm, Waters, Milford, USA) were used for clean-up sample matrix from buckwheat tea sample. Each analysis was performed in three replicates.

(II) *CE*

Separations were performed using a polyamide-coated, fused silica capillary (Polymicro technology, Phoenix, AZ, USA), 0.47 m length with 75 µm i.d. and a distance of 0.385 m from the point of injection to the detection window. The analyses were performed on a HP^{3D} CE (Agilent Technologies, Bracknell, UK), equipped with a positive power supply. The applied separation voltage was +20 kV. Temperature of the capillary tube during electrophoresis was maintained at 25°C. Analyte injection was carried out by using the pressure of 50 mbar and the injection time was 5 s. The electrophoretic zones were detected at 208 nm with a photodiode array detector. The background electrolyte was a mixture of 20 mM boric acid and 20 mM $Na_2B_4O_7$ at pH 9.4.

Before operation, the capillary was pre-treated by flushing with electrolyte for 5 min. Each analysis was performed in three replicates. The detection limits were determined using a signal-to-noise ratio of 3.

III. *Sample Preparation*

(I) *Buckwheat Tea*

Methanol (3 mL) was passed through a C18 cartridge followed by 10 mL of water. Buckwheat tea (5 mL) was then passed slowly through the C18 cartridge followed by 2 mL of water. Rutin was then slowly back-flushed from the C18 cartridge into a 5 mL volumetric flask with 3 mL methanol and made up to the volume with water. The sample solution was diluted 25 times with water before injected to HPLC and CE. Peak area of rutin was detected at 356 nm for HPLC and 208 nm for CE.

(II) *Fagopyrum tataricum Seeds*

Dried *Fagopyrum tataricum* seeds were ground and

sieved. Fifteen milliliters of methanol were added to triplicate of 0.05 g powdered seed. The sample was heated in water bath at 45°C for 30 min, following by ultrasonic bath at room temperature for 20 min. Then, it was transferred to a 25 mL volumetric flask and diluted to volume with water. The solution was filtered through a 0.45 μm membrane and diluted 5 times with water prior injection to HPLC and CE.

Table 1. An overview of analytical methods used for the determination of rutin

Sample	Method/Column (loop)	Detection	Detection limit (ng/mL)	Analysis time (retention or migration time), min	Refs.
Buckwheat *Fagopyrum esculentum* Moench	HPLC-C18 150 mm × 1.0 mm, 3 μm (5 μL)	Electrochemical detection	0.86	30 (30)	(2)
Chinese traditional drug	CE-40 cm with 25 μm i.d.	Electrochemical detection	263	9 (7)	(4)
Chinese herb medicines and human urine	CE-40 cm with 25 μm i.d.	Amperometric detection	122	20 (11.1)	(6)
Pueraria lobata (Wild.) Ohwi	CE-40 cm with 25 μm i.d.	Electrochemical detection	312	12 (11)	(7)
Grapefruit peel and juice	CE-75 cm with 25 μm i.d.	Electrochemical detection	180	22 (20)	(8)
Red wine	HPLC-C18 250 mm × 4.0 mm, 5 μm (50 μL)	360 nm	23.4	60 (9.8)	(12)
Sea buckthorn leaves	HPLC-C18 250 mm × 4.6 mm, 5 μm (10 μL)	257 nm	0.79	13 (5)	(13)
Human plasma	HPLC-C18 150 mm × 2.1mm, 5 μm (10 μL)	270 nm	-	15 (7)	(14)
Rat urine and chicken plasma	HPLC-C18 150 mm × 4.6mm, 5 μm (20 μL)	254 nm	30	25 (4.82)	(16)
Water	HPLC-C18 150 mm × 4.6 mm, 5 μm (10 μL)	327 nm	200	13 (12.3)	(17)
Buckwheat phenolic compound	HPLC-C18 150 mm × 4.6 mm, 1.8 μm (10 μL)	ESI-TOF-MS	310	70 (44.6)	(18)
Buckwheat dough	HPLC-C18 150 mm × 6 mm (10 μL)	370 nm	-	10 (6.5)	(19)
Plytycladus orientalis (L.) Franco	HPLC-C18 150 mm × 4.6 mm, 3.5 μm (10 μL)	356 nm	-	25 (6.1)	(20)
Solid oral dosage forms	HPLC-C18 150 mm × 4.6 mm, 5 μm (20 μL)	360 nm	2600	3 (2.3)	(21)
St. John's Wort	HPLC-C18 250 mm × 4.6 mm, 5 μm (10 μL)	270 nm	-	50 (13.7)	(22)
Hypericum perforatum leaves and flowers	CE-16 cm with 0.3 mm i.d.	254 nm	-	35 (33)	(23)
Herbal medicines	MEKC-50 cm with 50 μm i.d.	214 nm	1900	18 (9)	(24)
Ricinus communis Linn. leaves	CE-50 cm with 25 μm i.d.	Amperometric detection	488	10 (6)	(25)
Buckwheat	MEKC-70 cm with 75 μm i.d.	270 nm	2480	13 (9.5)	(26)
Achillea millefolium plant	CE-56 cm with 50 mm i.d. HPLC-C18 250 mm × 4.6 mm, 5 μm (12.5 μL)	335 nm 370 nm	700 900	20 (12.2) 30 (27)	(27) (27)

RESULTS AND DISCUSSION

I. *Choice of Optimal Absorption Wavelength of Rutin*

The spectra of rutin dissolved in methanol-acetate buffer at pH 4.1 and borate buffer at pH 9.4 were shown in Figure 2. The optimal wavelength of 356 nm for HPLC was selected due to maximum absorbance and no interference peak from methanol. For CE, 208 nm was selected as detection wavelength due to high absorbance without interference peak from background electrolyte.

II. *Separation Parameters*

(I) *Chromatogram*

Three mobile phase parameters affected the retention and peak area of rutin, namely the percentage of methanol, pH and concentration of acetate. Then, methanol over the range of 40 - 60%, pH 4.1 - 6 and acetate 5 - 15 mM were investigated. A mobile phase comprising 50% (v/v) methanol and 10 mM acetate buffer at pH 4.1 was selected due to provide short analysis time and high peak height. Figure 3A shows chromatogram of standard rutin with retention time 1.6 min under optimal condition. The developed HPLC method performed on monolithic RP 18 provided high sensitivity and total analysis time was shorter than all previous papers as shown in Table 1.

(II) *Electropherogram*

Four parameters affected the migration time and peak height of rutin, namely concentration of sodium borate and boric acid, pH of electrolyte, apply potential and injection time. Concentration of sodium borate and boric acid over the range of 10 - 30 mM salt and acid, pH of electrolyte from 8.8 to pH 10, positive applied potential between 10 and 30 kV and injection time from 5 to 10 s were investigated. The background electrolyte containing 20 mM sodium borate and 20 mM boric acid at pH 9.4 was selected due to short migration time and high peak height. The 20 kV applied potential and injection time at 5 s were optimal condition which yielded the highest peak height and lowest peak width. Electropherogram of standard rutin with migration time of 5.6 min under optimal condition showed in Figure 3B. By comparison, the developed CE provided higher sensitivity and faster analysis time than the previous works as shown in Table 1.

III. *Analytical Characteristic Performance*

The detection limit of rutin (determined at a signal-to-noise ratio of 3), the linearity correlation obtained from

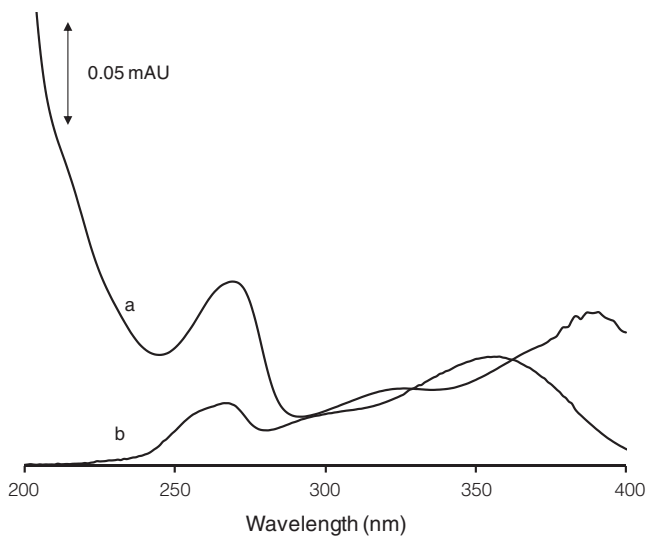

Figure 2. Absorption spectra of (a) standard rutin in borate buffer at pH 9.4 and (b) standard rutin in methanol-water (50 : 50, v/v) containing 10 mM acetate buffer at pH 4.1.

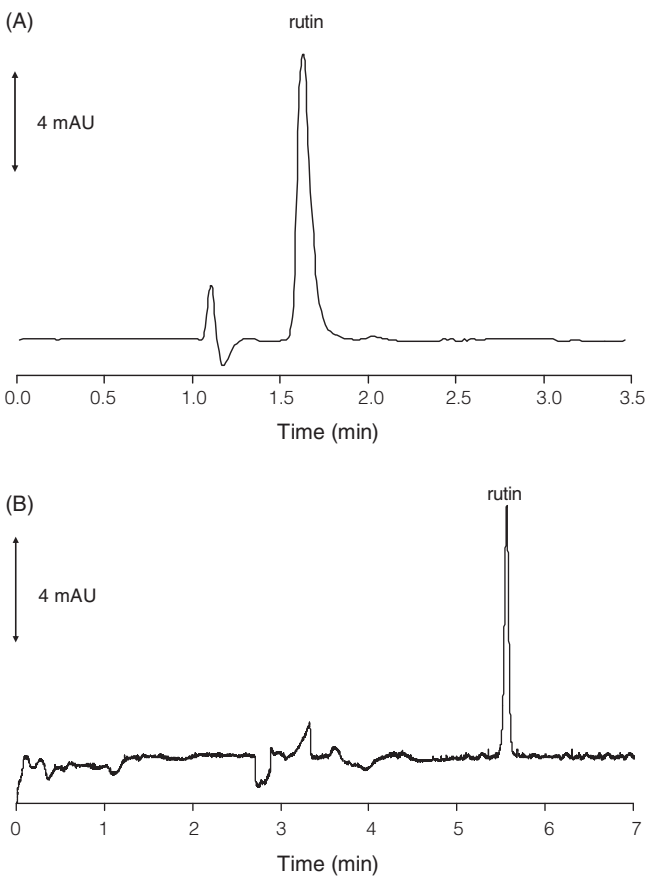

Figure 3. (A) Chromatogram of 10 mg/L standard rutin in mobile phase containing methanol-water (50 : 50, v/v) and 10 mM acetate buffer at pH 4.1. Separation column: monolithic column C18 (100 × 4.6 mm i.d.); injection loop: 5 μL; flow rate: 1.5 mL/min; temperature 30°C; detection by absorbance at 356 nm. (B) Electropherogram of standard rutin (10 mg/L), Conditions: column: bare fuse silica 47 cm; electrolytes: 20 mM H_3BO_3 and 20 mM $Na_2B_4O_7$ at pH 9.4; applied voltage: +20 kV; injection time: 5 sec; temperature: 25°C; detection by absorbance at 208 nm.

external calibration curve up to at least 80 μg/mL and % relative standard deviation (%RSD) for elution time and peak area showed in Table 2. The results showed the sensitivity obtained from HPLC higher than CE. By comparison, the sensitivity of the developed HPLC method was also higher than previous CE works. In addition, the sensitivity of the developed CE was higher than previous CE works and the analysis time of both developed methods was faster than all previous HPLC and CE works as shown in Table 1.

IV. *Extraction of Rutin from Samples*

(I) *Buckwheat Tea*

The buckwheat tea required a clean up step to reduce its ionic strength. The clean up required methanol back flush to elute the bound rutin from C18 cartridge. Rutin could bind to the adsorbent by the hydrophobic interactions, while much of the sample matrix passed through the cartridge. The interstitial sample matrix trapped in the cartridge could be removed by slowly flushing with 2 mL water. Then, 3 mL methanol was used to remove rutin completely from C18 by back-flushed. The resulting effluent was diluted to 60% methanol in the final solution with water before injecting to HPLC and CE.

The recovery of rutin from clean up step was determined by comparison of the peak area of rutin obtained from a standard rutin with buckwheat tea, and mixture of rutin and buckwheat tea obtained after elution from a C18 cartridge. The same sample solution was used for HPLC and CE methods and the recoveries of rutin were 105.0% and 100.0%, respectively.

(II) *Fagopyrum tataricum Seeds*

Rutin containing in *Fagopyrum tataricum* seeds was extracted by using methanol, heat and sonication. Amounts of methanol over the range of 10 - 20 mL, heating time in water bath between 30 and 60 min, and time for ultrasonic bath over the range of 10 - 30 min were investigated in order to obtain the highest yield of rutin. The optimal condition for the extraction was 15 mL methanol, 30 min for heating in water bath and 20 min for sonication. The recovery of rutin in *Fagopyrum tataricum* seeds by HPLC and CE was 99.6% and 98.8%, respectively.

V. *Determination of Rutin in Samples*

(I) *Buckwheat Tea*

A preliminary experiment found sample matrix in buckwheat tea reduced peak height and peak area of HPLC and CE methods. The sample matrix was removed by using Sep-Pak C18 cartridge and rutin was then eluted from C18 cartridge by back flushing with methanol. External calibration curve

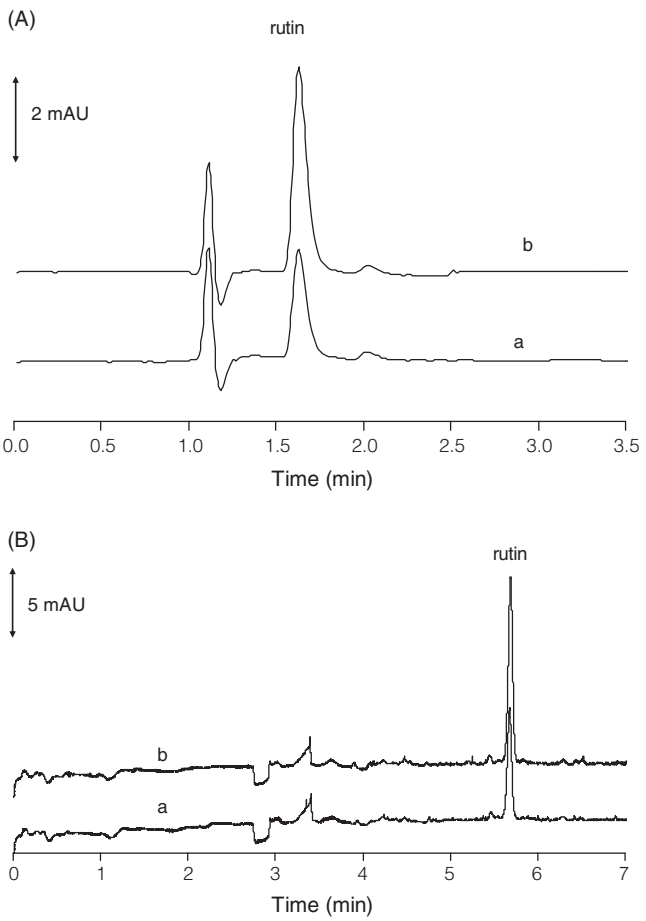

Figure 4. (A) Chromatograms of (a) buckwheat tea and (b) buckwheat tea spiked with 2 mg/L of rutin. Chromatographic conditions were identical to those outlined in Figure 3A. (B) Electropherogram of (a) buckwheat tea and (b) buckwheat tea spiked with 5 mg/L of rutin. Electropherogram conditions were identical to those outlined in Figure 3B.

Table 2. Comparable performance characteristics of rutin analysis by HPLC and CE methods

Method	Linearity correlation	Detection limit (ng/mL)	% RSD (n = 5)				Analysis time (retention time or migration time), min
			Retention time or migration time		area		
			Intra-day	Inter-day	Intra-day	Inter-day	
HPLC	0.9990	23.91	0.199	0.206	0.832	0.840	1.6
CE	0.9992	55.67	0.377	0.385	0.963	0.992	5.6

was used to determined rutin in sample by HPLC and CE. Chromatogram and electropherogram of buckwheat tea were shown in Figure 4A and Figure 4B. The rutin peak was well resolved from other components present in sample, and the spiking sample with standard rutin was used to confirm the peak identity (Figure 4B). The result obtained from HPLC was 63.13 μg/mL, which agreed well with those from CE (61.04 μg/mL), whilst the labeled concentration of rutin on buckwheat tea bottle was 60 μg/mL.

(II) *Fagopyrum tataricum Seeds*

Chromatogram and electropherogram of *Fagopyrum tataricum* seeds are shown in Figure 5A and Figure 5B, respectively. The result obtained from HPLC was 5231 μg/mL, which agreed well with that from CE value (5204 μg/mL). Figure 5B showed matrix in *Fagopyrum tataricum* seeds made migration time of rutin from *Fagopyrum tataricum* seed sample shorter than standard rutin (Figure 3B).

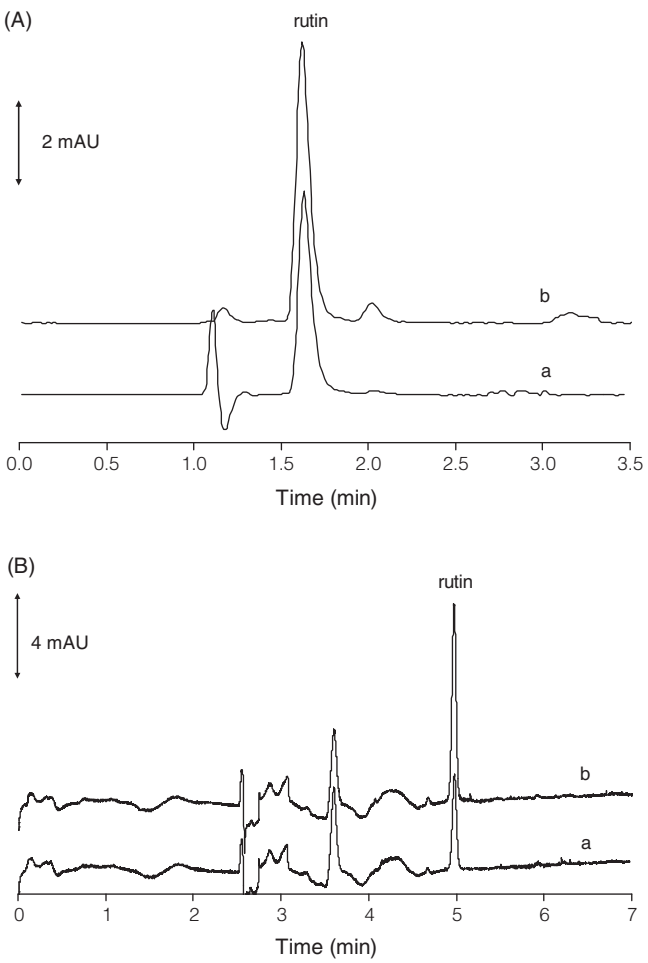

Figure 5. (A) Chromatograms of (a) standard 5 mg/L of rutin and (b) *Fagopyrum tataricum* seeds. Chromatographic conditions were identical to those outlined in Figure 3A. (B) Electropherogram of (a) *Fagopyrum tataricum* seeds and (b) *Fagopyrum tataricum* seeds spiked with 2 mg/L of rutin. Electropherogram conditions were identical to those outlined in Figure 3B.

Advantages of the proposed CE method for the determination of rutin was without using organic solvent compared to HPLC. For HPLC method, the advantages were shorter analysis time and lower detection limit than CE. However, both developed methods provided simple sample preparation, fast analysis time, high sensitivity and no interference peak for rutin determination in buckwheat tea and *Fagopyrum tataricum* seeds.

CONCLUSIONS

The results demonstrated that both developed HPLC and CE methods were simple, fast and sensitive for the determination of rutin in buckwheat tea and *Fagopyrum tataricum* seeds. The extraction of rutin from *Fagopyrum tataricum* seeds and the clean up sample matrix from buckwheat tea were simple, fast and provided good recovery. Based on the comparison, the concentration of rutin obtained from HPLC agreed well with the value from CE in both samples.

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