Separation and Determination of Rutin in Apples by High Performance Liquid Chromatography

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Abstract

Rutin is a flavonol glycoside and its main function in the body is to help the body strengthen arteries and veins, and harden bones and teeth. It is found in many fruits such as in apples. The aim of this research work is to develop a simple and fast high performance liquid chromatography (HPLC) method for the determination of rutin. The separation of rutin by HPLC was performed using a monolithic C18 column at detected wavelength, 356 nm. The optimal composition of mobile phase was methanol-water (40-60 v/v) in 10 mM acetate buffer. This condition provided a retention time of rutin at 2.92 min. The limit of detection (LOD) was 0.32 µg/mL (sample loop=5 µL), and %RSD (n=5) of retention time, peak area, and peak height were 0.052, 0.635, and 0.578, respectively. This method has been successfully applied for the determination of rutin in apple juices. The results showed the highest concentration of rutin was found in green (14.3 µg/mL), followed by Fuji (5.7 µg/mL) and red delicious (1.4 µg/mL) apples, respectively.

Keywords: rutin, apple, high performance liquid chromatography

1. Introduction

Rutin is found in many plants, fruits, and vegetables (Structure is shown in Fig.1). There are indications that rutin can inhibit some cancerous and precancerous conditions. In addition, rutin can inhibit platelet aggregation, thus making the blood thinner, and improve circulation. Moreover, rutin has been used clinically as a therapeutic medicine because of its strong antioxidant properties [1]. With several advantages of rutin for human health, therefore, the development of a simple and fast method for the determination of rutin in food samples without an interference peak, is necessary.

High performance liquid chromatography [2-10] and capillary electrophoresis [11-17] were the commonly used methods for the determination of rutin by using spectrophotometric and electrochemical detectors. Those methods were developed for many compounds of interest in samples. The methods are time-consuming (Table 1). A small number of papers were directed toward the detection of rutin in samples. For example, the determination of rutin in red wine on RP-HPLC was developed on bonded phase column with a limit of detection of 23.4 ng/mL (injection time=50 μL) at retention time of 9.8 min, and using 60 min for analysis time [2]. The aim of this research is to reduce analysis time for the determination of rutin in apple juices by a fast HPLC method on monolithic RP 18 column.

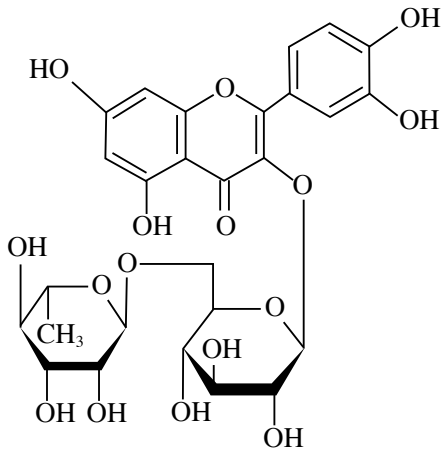

Fig. 1. Structural formula of rutin.

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

Sample	*Method/Column (loop)*	*Detection*	*Detection limit, (ng/mL)*	*Analysis time (min)*	*Refs.*
Apple	HPLC-monolithic C18 100 mm x 4.6 mm(5 μL)	356 nm	320	4	Present work
Red wine	HPLC-C18 250 mm x 4.0 mm, 5μm (50 μL)	360 nm	23.4	60	2
Buckwheat Fagopyrum esculentum Moench	HPLC-C18 150 mm x 1.0 mm, 3μm (5 μL)	Electrochem. detection	0.86	30	3
Sea buckthorn leaves	HPLC-C18 250 mm x 4.6 mm, 5μm (10 μL)	257 nm	0.79	13	4
Human plasma	HPLC-C18 150 mm x 2.1mm, 5μm (10 μL)	270 nm	-	15	5
Pueraria lobata (Wild.) Ohwi	CE-40 cm with 25 μm i.d.	Electrochem. detection	312	12	7
Hypericum perforatum leaves and flowers	CE-16 cm with 0.3 mm i.d.	254 nm	-	35	8
Chinese herb medicines and human urine	CE-40 cm with 25 μm i.d.	Amperometric detection	122	20	9
Chinese traditional drug	CE-40 cm with 25 μm i.d.	Electrochem. detection	263	9	10

Sample	*Method/Column (loop)*	*Detection*	*Detection limit, (ng/mL)*	*Analysis time (min)*	*Refs.*
Grapefruit peel and juice	CE-75 cm with 25 μm i.d.	Electrochem. detection	180	22	11
Rat urine and chicken plasma	HPLC-C18 150 mm x 4.6mm, 5 μm (20 μL)	254 nm	30	25	12
Herbal medicines	MEKC-50 cm with 50 μm i.d.	214 nm	1900	18	13
Ricinus communis Linn. leaves	CE-50 cm with 25 μm i.d.	Amperometric detection	488	10	14
Water	HPLC-C18 150 mm x 4.6mm, 5 μm (10 μL)	327 nm	200	13	15
Buckwheat phenolic compound	HPLC-C18 150 mm x 4.6mm, 1.8 μm (10 μL)	ESI-TOF-MS	310	44	16
Buckwheat dough	HPLC-C18 15 cm x 6 mm (10 μL)	370 nm	-	10	17

2. Materials and Methods

2.1 Chemicals and Samples

A stock solution of rutin (Wako Pure Chemical Industries, Osaka, Japan) was prepared by dissolving 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. HPLC methanol (Merck, Darmstadt, Germany) and acetic acid (BDH, Poole, UK) were used. Red delicious, Fuji, and green apples were bought from a supermarket.

2.2 Instrumentation

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) was used as the analytical column and was fitted with a Chromolith RP-18 (4.6 mm i.d., 5 mm, Merck) guard column. The mobile phase was methanol-water (40-60% 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.

2.3 Sample Preparation

A juicer was used to separate apple juice from residue. The mixture was left undisturbed until the apple juice was clearly separated from residue. Apple juice was then filtered with a 0.45 μm filter. Fuji and green apple juice were diluted 5 times, while red delicious juice was used without dilution, before injecting into an HPLC. Each analysis was performed using three replicates.

3. Results and Discussion

3.1 Choice of Optimal Absorption Wavelength of Rutin

The spectrum of rutin dissolved in methanol-water (40:60 v/v) in acetate buffer at pH 4.1 is shown in Fig. 2. The optimal wavelength of 356 nm for HPLC was selected due to this wavelength providing maximum absorbance.

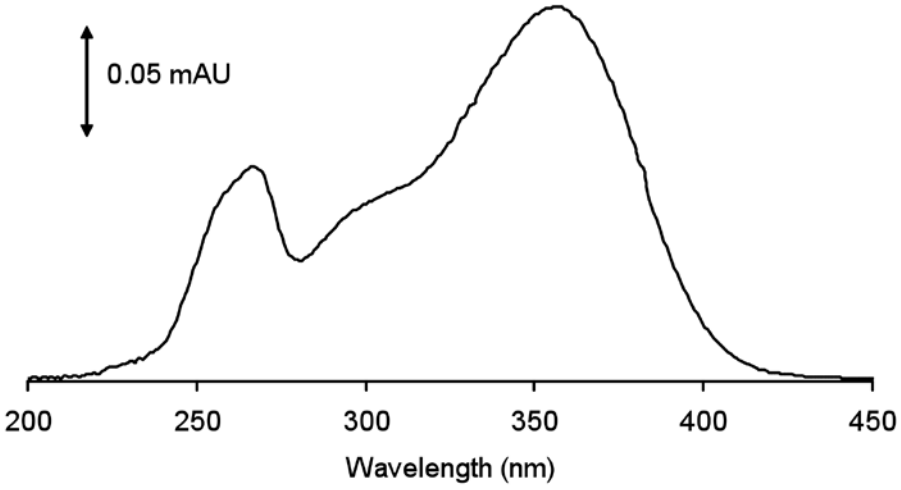

Fig. 2. Absorption spectra of standard rutin in methanol-water (40:60, v/v) containing 10 mM acetate buffer at pH 4.1.

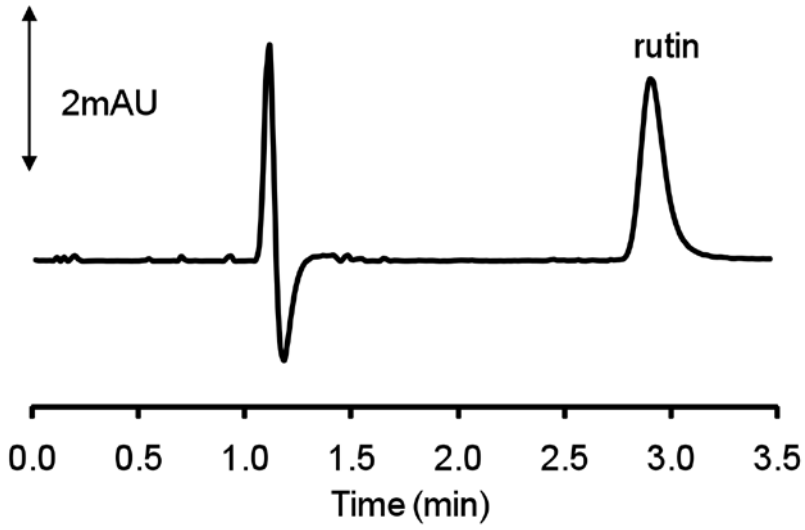

Fig. 3. Chromatogram of 5 mg/L standard rutin in mobile phase containing methanol-water (40:60, v/v) in 10 mM acetate buffer at pH 4.1. Separation column: monolithic column C18 (100 x 4.6 mm I.D.); injection loop: 5 μL; flow rate: 1.5 mL/min; temperature 30°C; detection by absorbance at 356 nm.

3.2 Separation Parameters

Three mobile phase parameters affected retention time, peak height and interference peak of rutin in apple juices, namely the percentage of methanol, pH, and concentration of acetate. Samples with methanol over the range 40-60%, pH 4.1-6, and acetate 5-15 mM, were investigated. A mobile phase comprising 40% (v/v) in 10 mM acetate buffer at pH 4.1 was selected as the optimal conditions for the determination of rutin due to providing short analysis time, high peak height, and no interference peak at retention time of rutin (2.92 min) in apple juices. Fig. 3 shows a chromatogram of 5 mg/L standard rutin at optimized conditions.

3.3 Analytical Characteristic Performance

The detection limit (determined at a signal-to-noise ratio of 3) of rutin was 0.32 μg/mL (sample loop=5 µL). Percent RSD (intra-day, n=5) of retention time and peak area were 0.052 and 0.635, respectively. For inter-day (n=5), % RSD of retention time and peak area were 0.074 and 0.840, respectively. The linearity correlation obtained from an external calibration curve was up to at least 80 µg/mL (r^2=0.9990).

3.4 Determination of Rutin in Apples

Rutin contained in red delicious, Fuji, and green apple juice was investigated on monolithic column C18 by the developed HPLC method. A preliminary experiment showed there was no interference peak at the retention time of rutin in apple juice, and no sample matrix was found when using a mobile phase containing 40% (v/v) in 10 mM acetate buffer at pH 4.1. Therefore, an external calibration curve was used to determine rutin in apple juices. Fig. 4 shows chromatograms of rutin in red delicious, Fuji, and green apple juices. The results show that the highest concentration of rutin was found in green (14.3 µg/mL), followed by Fuji (5.7 µg/mL), and red delicious (1.4 µg/mL) apples.

Advantages of the proposed HPLC method are simple sample preparation, and faster analysis time compared to Refs. in Table 1. There was no interference peak at the retention time of rutin in apple juices, and only 4 min was used for analysis time.

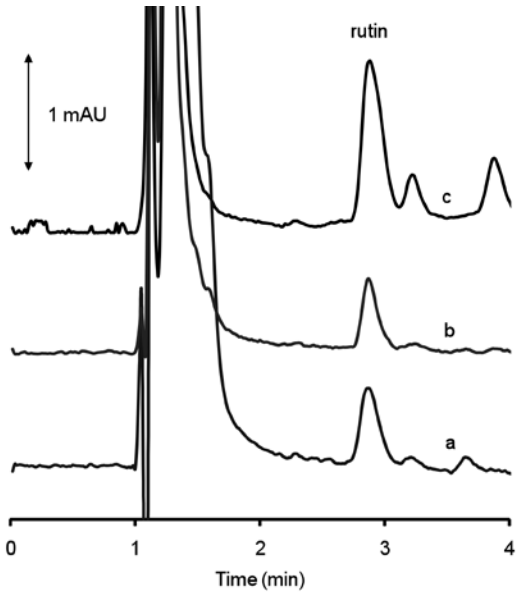

Fig. 4. Chromatograms of (a) red delicious apple juice; (b) Fuji apple juice diluted 5 times in water; and (c) green apple juice diluted 5 times in water. Mobile phase was methanol-water (40:60, v/v) containing 10 mM acetate buffer at pH 4.1. Separation column: Chromolit column RP-18 (4.6 mm i.d., 100 mm) injection loop: 5 μL; flow rate: 1.5 mL/min; temperature 30 °C; detection by absorbance at 356 nm.

4. Conclusion

The result demonstrated that the developed HPLC method for the determination of rutin in apple juices was simple and fast. The sensitivity of HPLC was satisfactory for the determination of rutin in apple juice samples without an interference peak.

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