Food Anal. Methods (2017) 10:83–91
DOI 10.1007/s12161-016-0552-0

Simultaneous Determination of Quercetin, Rutin, Naringin, and Naringenin in Different Fruits by Capillary Zone Electrophoresis

Almas F. Memon[1] · **Amber R. Solangi**[1] · **Saima Q. Memon**[2] · **Arfana Mallah**[2] · **Najma Memon**[1] · **Ayaz A. Memon**[1]

Received: 9 February 2016 / Accepted: 25 May 2016 / Published online: 10 June 2016

Abstract A fast and reliable capillary zone electrophoretic (CZE) method has been developed for the simultaneous determination of four fruit flavonoids using photodiode array (PDA) detector. The effects of CE parameters including concentration and pH of the running buffer, voltage, and injection time were optimized. Under the optimized conditions, all flavonoids were well determined in a 10 mM borate buffer of pH 8.5 within 10 min at an applied voltage of 25 kV. Naringin, naringenin, and quercetin were found to have linear response in the range of 3.12–200 μg/mL whereas rutin's response was linear from 6.25 to 200 μg/mL. LOD was found to be 0.406, 0.314, 0.582, and 0.333 μg/mL and LOQ 1.355, 1.046, 1.941, and 1.11, respectively. Relative standard deviations (RSD) were found to be less than 3 % for both migration time and peak height which shows long-term stability and good reproducibility of the developed method. The method was successfully applied for the simultaneous determination of flavonoids from various fruit juice samples.

Keywords CE · Flavonoids · Naringin · Naringenin · Quercetin and rutin in fruits

✉ Amber R. Solangi
ambersolangi@gmail.com

[1] National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

[2] Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan

Introduction

Flavonoids consist of large family of more than 4000 ubiquitous secondary plant metabolites and can further be divided into five subclasses: flavonols, flavones, flavanones, anthocyanins, and catechins (Wang and Huang 2004). Most of the edible vegetables and fruits contain flavonoids, but their type varies according to the dietary sources they are obtained from (Awad et al. 2000), e.g., flavanones and flavones are usually found in the same plant, yet flavones and flavonols are generally not present together, nor are flavanones and anthocyanins (Merken and Beecher 2000). The most common flavonol in the diet is quercetin (3,3′,4′,5,7-pentahydroxy flavone) (Ignat et al. 2011). It is present in plants in glycosidic form with quercetin-3-rutinoside, and also called rutin (Erlund 2004). Over the past years, both quercetin and rutin have gained tremendous interest, up to the present, rutin has been used clinically as the therapeutical medicine, and over 130 preparations containing quercetin or rutin are registered as drugs worldwide. Flavanones occur almost exclusively in citrus fruits. The highest concentrations are found in the solid tissues, but concentrations of several hundred milligrams per liter are present in the juice as well (Awad et al. 2000). Naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) is a flavanone present in many citrus fruits while naringenin is the aglycone of naringin (Fang et al. 2006).

Beneficial effects of regular consumption of fruits and vegetables are associated with high content of flavonoids present in them (Lotito and Frei 2004). Their dietary significance exhibits a wide range of biological effects, including anti-oxidative, anti-microbial, anti-bacterial, anti-ulcer, anti-mutagenic, anti-carcinogenic, anti-inflammatory, anti-aging, anti-allergic, anti-thrombic, anti-viral, anti-neopasic, anti-tumor,

vasodilatory, and cardioprotective actions (Biesaga 2011; Gil and Couto 2013 ; Awad et al. 2000; Volikakis and Efstathiou 2005; Gattuso et al. 2007).

Ziziphus mauritiana L. belongs to the Rhamnaceae family, fruit of which is commonly known as "ber" in India and Pakistan (Memon et al. 2012). The three cultivated varieties of *Z. mauritiana* L. in Pakistan (particularly in Sindh Province) are locally known as Gola ber (var. Gola Lemai, Gola Achri, Gola Lootri, Gola Dehli), Soofi ber, and Kheerol (Chambeli) ber (Memon et al. 2013). Ber fruit is consumed fresh or dried and is used to prepare jams, candied fruit, beverages, and other food products (Memon et al. 2012). Ber fruit is known to contain a wide array of phytochemicals and minerals such as amino acids, carbohydrates, ascorbic acid, flavonoids, phenolic acids, vitamins A and C, phosphorus, and calcium (Memon et al. 2013).

Several analytical methods have been described for the separation and determination of flavonoids using various techniques, such as TLC (Males and Medic 2001; Vogel et al. 2005), HPLC (Wang and Huang 2004; Ishii et al. 2003; Pascual et al. 1998), HPLC-MS (Lin et al. 2000; Wu et al. 2004; Sanchez et al. 2003), GC-MS (Fiameogos et al. 2004; Deng and Zito 2003) and Capillary Electrophoresis (CE) (Akiyama et al. 2000; Bo et al. 2002). Compared with conventional chromatographic methods, capillary electrophoresis (CE) has many valuable advantages, including excellent separation efficiency, high resolution, short analysis time, easy automation, and low solvent and sample consumption.

Flavonoids have been analyzed by different modes of CE. A micellar electrokinetic capillary chromatographic (MEKC) method was developed and validated for the simultaneous determination of flavonoids (Ganzera et al. 2008). A selective and sensitive microemulsion EKC (MEEKC) method with electrochemical detection has been developed for separation and determination of flavonoids (Yu et al. 2008). Among different modes in CE separation, capillary zone electrophoresis (CZE), using an uncoated capillary column requiring less maintenance is the most frequent and simplest separation mode, which makes the optimization of conditions easy (Liu et al. 2006). A number of papers are reported every year using CZE for the separation and determination of flavonoids from various matrices. For example, Liu et al. (Liu et al. 2006) developed a CZE method coupled with diode-array detection for simultaneous determination of four flavonoids including icariin, epimedin A, epimedin B, and epimedin C in *Epimedium*, in which 50 mM borate buffer containing 22 % acetonitrile was used at pH 10 at 15 kV. Wu et al. (Wu et al. 2007) determined five flavonoids (hesperidin, naringin, hesperedin, naringenin, and rutin) and ascorbic acid in grapefruit peel and juice by CE-ED, using 60 mM borate buffer of pH 9 and analytes were well separated within 25 min. Zhang et al. (Zhang et al. 2008) simultaneously determined six flavonoids including apigenin, luteolin, kaempferol, quercetin, (+)-catechin, (−)-epicatechin in chrysanthemum by CZE with amperometric detection (CZE-AD), where β-cyclodextrin and the mixture of methanol and ethanol were used as running buffer modifiers and the baseline separation of six analytes was achieved in 20 min by using 36 mM borate-phosphate buffer of 8.8 pH at 18 kV. Ren et al. (Ren et al. 2009) developed a CZE method for the simultaneous determination of nine flavonoids in *Anaphalis margaritacea*, in which 25 mM $Na_2B_4O_7$ and 10 mM NaH_2PO_4 buffer solution was used at pH 9.6 at 20 kV. Zhang et al. (Zhang et al. 2010) simultaneously determined flavonoids and anthraquinones (emodin, kaempferol, apigenin, luteolin, and rhein) in chrysanthemum by CE-AD, where all five analytes were well separated within 17 min, using borate-phosphate buffer of pH 9 at 19 kV.

The purpose of this work is to develop a simple, fast, effective, and reliable CZE method for simultaneous determination of four flavonoids including two flavonols (quercetin and rutin) and two flavanones (naringin and naringenin) in different fruits such as apples (*Malus domestica*), oranges (*Citrus sinenis*), grapefruits (*Citrus paradisi*), and ber (*Z. mauritiana* L.), using photodiode array (PDA) detector. To the best of our knowledge, no one method is reported to describe the simultaneous determination of these four flavonoids in different fruit samples.

Experimental

Standard and Reagents

Naringin, naringenin, ethyl alcohol, and hydrochloric acid were purchased from Sigma-Aldrich (China and UK). Quercetin dihydrate and rutin were purchased from Fluka (Switzerland). Boric acid and sodium hydroxide were purchased from E. Merck (Germany). Sodium borate decahydrate was obtained from Daejung Chemicals (Korea). Methanol was purchased from Fischer Chemicals (UK). All reagents were HPLC grade and used without further purification. Double-deionized water was used throughout the experiments.

Stock solutions of flavonoids: naringin, naringenin, rutin, and quercetin were prepared separately by dissolving 0.1 g of each flavonoid in 100 mL of methanol. The background electrolyte (BGE) for separation was 10 mM borate buffer (pH 8.5) which was prepared by dissolving the appropriate amounts of sodium borate decahydrate and boric acid in double-deionized water. The pH of the background buffer was adjusted with either 0.1 M NaOH or 0.1 M HCl. All solutions were filtered through 0.45-μm cellulose acetate filter paper prior to use for CE experiments.

Samples Collection

Fresh fruits of apple, orange, grapefruit, and four species of ber (Soofi ber, Kheerol ber, Gola Lemai ber, and Lootri ber) were purchased from local market of Hyderabad and commercial juices of apple and orange were purchased from superstores of Hyderabad.

Fresh fruits of apples, oranges, and grapefruit were treated in a commercial home juicer and juice was collected, whereas the ber fruit was washed with tap water and pulp was separated from its seed. The edible pulp part of the fruit was cut into small pieces and freeze dried. After drying, the samples were ground into a fine powder and stored in a freezer until analyzed.

Instrumentation and Electrophoretic Conditions

All the experiments were conducted with a Beckman P/ACE MDQ CE instrument (Beckman Coulter Inc., USA) equipped with a photodiode array detector with 32 Karat Software. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix) with dimensions of 57 cm total length, 50 cm effective length, 75 μm inner diameter, and 375 μm outer diameter were used. Capillary was conditioned and regenerated as the previously reported method (Memon et al. 2015).

Samples were injected by means of the autosampler using hydrodynamic method (0.5 psi). An injection time of 10 s was used for all analyses. The separation voltage was 25 kV while the temperature of capillary was maintained at 25 °C. The pH of solutions was measured using an inoLab pH meter.

Sample Preparation

Samples were prepared by a reported method (Wu et al. 2007) with some modifications. Ber flavonoids were extracted by keeping mixture of 1 g of freeze-dried ber samples and 10 mL of ethyl alcohol in an ultrasonic bath for 2 h. The extract was then filtered through a filter paper (Whatman # 42) and the extraction procedure was repeated thrice. After filtration through 0.45-μm cellulose acetate filter paper, 80 μL of sample was diluted with 10 mM borate buffer to 1 mL and the clear solution was injected in CE to record electropherogram.

Juice samples of apple, orange, and grapefruit were separately centrifuged at 5000 rpm for 10 min; then the juice was filtered through 0.45-μm cellulose acetate filter paper; then 80 μL samples was diluted with 10 mM borate buffer to 1 mL; and the clear solution was run for recording electropherogram.

Results and Discussion

Optimization of the Method

The mixture of four flavonoids was analyzed by an optimized CZE method. All four flavonoids are soluble in methanol and show appreciable absorbance at 214 nm. Separation was optimized with respect to the pH and concentration of the background buffer. The separation voltage and injection time were also optimized to resolve the standard mixture of all four flavonoids in shortest time.

Buffer Selection

Sodium hydrogen phosphate (pH 7.5–9.0), sodium acetate (pH 6–7) and sodium tetraborate (pH 8–10) buffers were tried to obtain the optimum CE conditions. Sodium tetraborate was found to have better efficiency in terms of peak shape, peak height, and shorter migration time. Therefore, it was selected and further optimized.

Effect of Buffer Concentration

The concentration of buffer is an important parameter in CE. The effect of buffer concentration in the range of 10–50 mM in intervals of 10 mM was studied. Figure 1 shows the effect of buffer concentration on migration time, peak area, and resolution. Migration time of the four analytes was increased by increasing buffer concentration due to the strong interaction of borate and flavonoids at higher buffer concentration. Peak area was also increased by increasing the concentration of the buffer since the interaction of borate ions and flavonoids considerably enhances UV absorbance of the analytes (Luo et al. 2007). The increase of migration time is because of decreasing electrosmotic flow (EOF) since the buffer concentration acts directly on the magnitude of the EOF, the higher the concentration lowers the EOF and vice versa, while low EOF produces a long migration time, attributed to the better resolution (Ren et al. 2009). Considering migration time, resolution, and peak area, the best separation was achieved at 10 mM borate buffer. Therefore, 10 mM was selected for further studies.

Effect of Buffer pH

In CE the migration time and separation of analytes are greatly affected by pH of buffer solution as it affects the zeta potential, the EOF, as well as the overall charge of the analytes (Xu et al. 2005a, 2005b). Therefore, the effect of buffer pH was studied from 8–10 in increments of 0.5, while keeping the concentration of buffer at 10 mM. Figure 2 indicates the increase in migration time of analytes with increasing pH; the phenomenon may be attributed to the dissociation of flavonoid

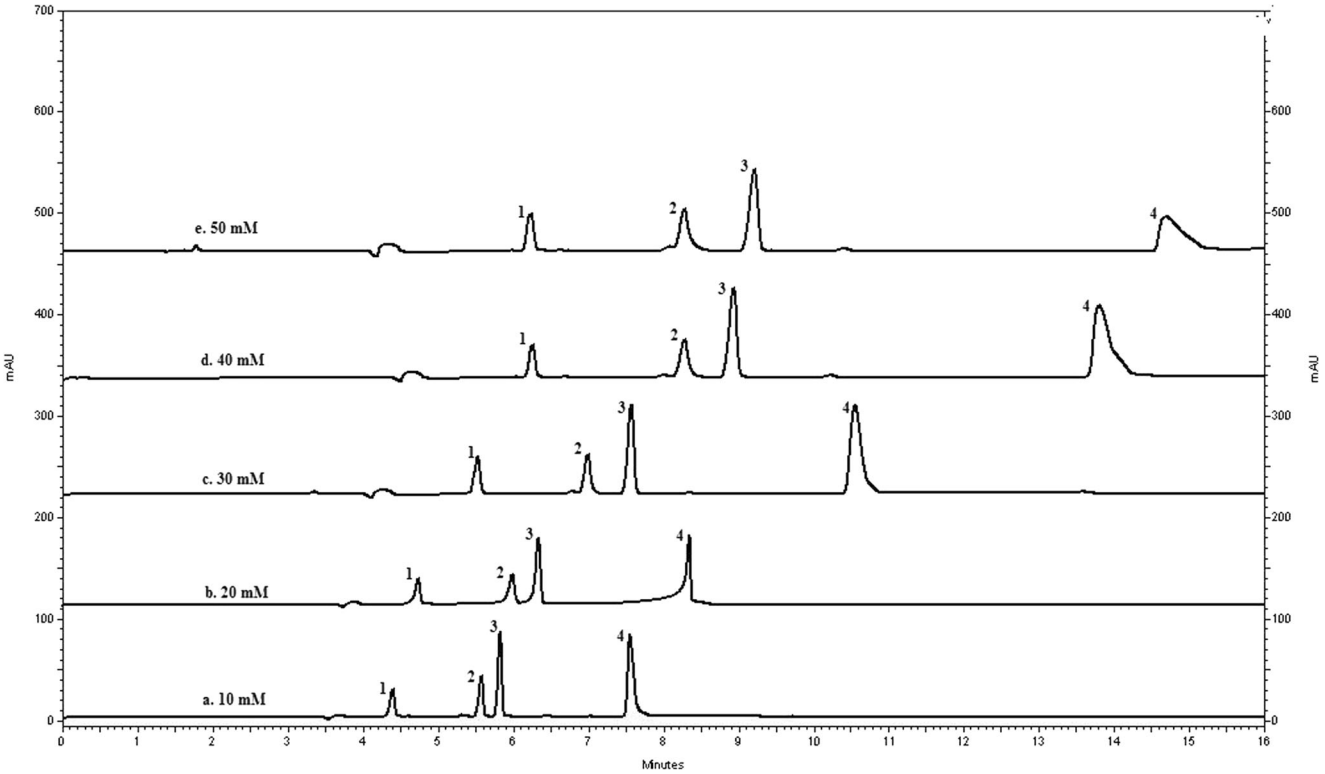

Fig. 1 Effect of buffer concentration on the migration behavior of analytes. CE conditions: 25 °C, 25 kV, and detection at 214 nm. Peak identification: *1* naringin, *2* rutin, *3* naringenin, *4* quercetin

hydroxyl groups with rising pH value (Xu et al. 2005a, 2005b). Moreover, at pH 8, the signal peaks of four analytes were very weak. At pH 9, quercetin was not detected and at pH 9.5 and 10, there was a large increase in migration time;

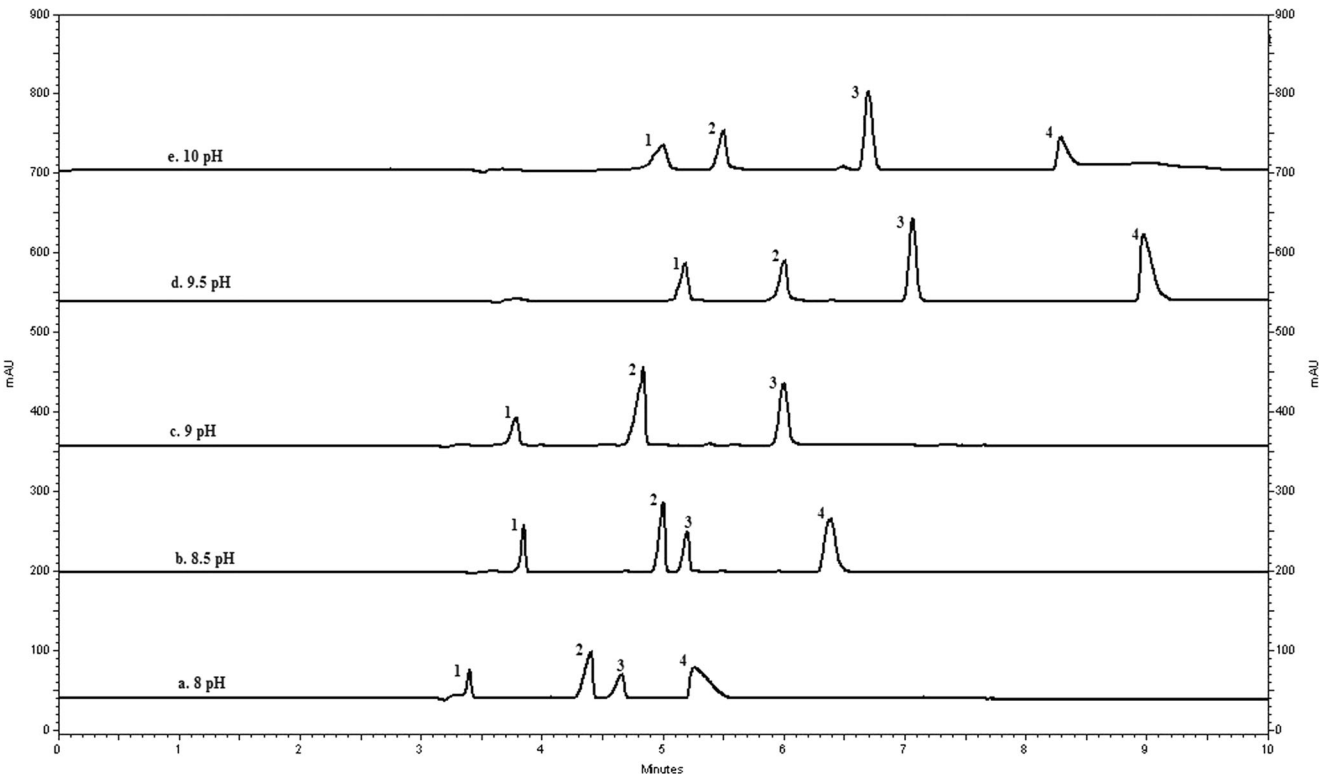

Fig. 2 Effect of buffer pH on the migration behavior at 10 mM concentration of borate. Other conditions and peak identification are shown in Fig. 1

therefore, borate buffer of pH 8.5 was selected because of reasonable migration time, better separation, greater absorbance, and fairly good peak shapes.

Effect of Applied Voltage and Injection Time

The effect of applied voltage was examined in the range of 10–30 kV with an interval of 5 kV, under the selected conditions. As the applied voltage has a negative relationship with migration time of the analytes, with increasing applied voltage a decrease in migration time of analytes was recorded but baseline noise increased and results in poor detection limits. As the voltage is increased, there is more joule heat generated in the capillary, which can be unfavorable to the separation because it led to broader peaks, which possibly results sample decomposition, or the formation of the bubbles inside the capillary. The better the heat is dissipated by the equipment, the higher the voltage that can be used (Pancorbo et al. 2004). In the present study, 25 kV was selected as the optimum applied voltage in terms of shorter analysis time, current intensity, and high separation efficiency.

Samples were injected into the capillary by hydrodynamic method and the effect of injection time was investigated by changing it from 4–12 s, at intervals of 2 s, at a constant pressure of 0.5 psi, and voltage of 25 kV. It was observed that by increasing injection time, peak current was increased, and peaks were broadened as injection time affects both the peak current and the peak shape (Xu et al. 2005a). When the injection time exceeded 10 s, the peak current increased and peak broadening became more severe. Therefore, considering resolution and sensitivity, 10 s was selected as the optimum injection time.

Figure 3 shows the typical electropherogram for standard mixture solution at the final optimized conditions as running buffer 10 mM borate buffer (pH 8.5), applied voltage 25 kV, hydrodynamic injection (10 s at 0.5 psi), and capillary temperature 25 °C. Baseline separation for all four flavonoids has been achieved within 10 min.

Validation of the Method

Linearity

The calibration curves were plotted (concentration vs. peak area) in the concentration range from 3.125 to 200 μg/mL for naringin, naringenin, and rutin, whereas 6.25 to 200 μg/mL for quercetin. The response was linear with coefficient of

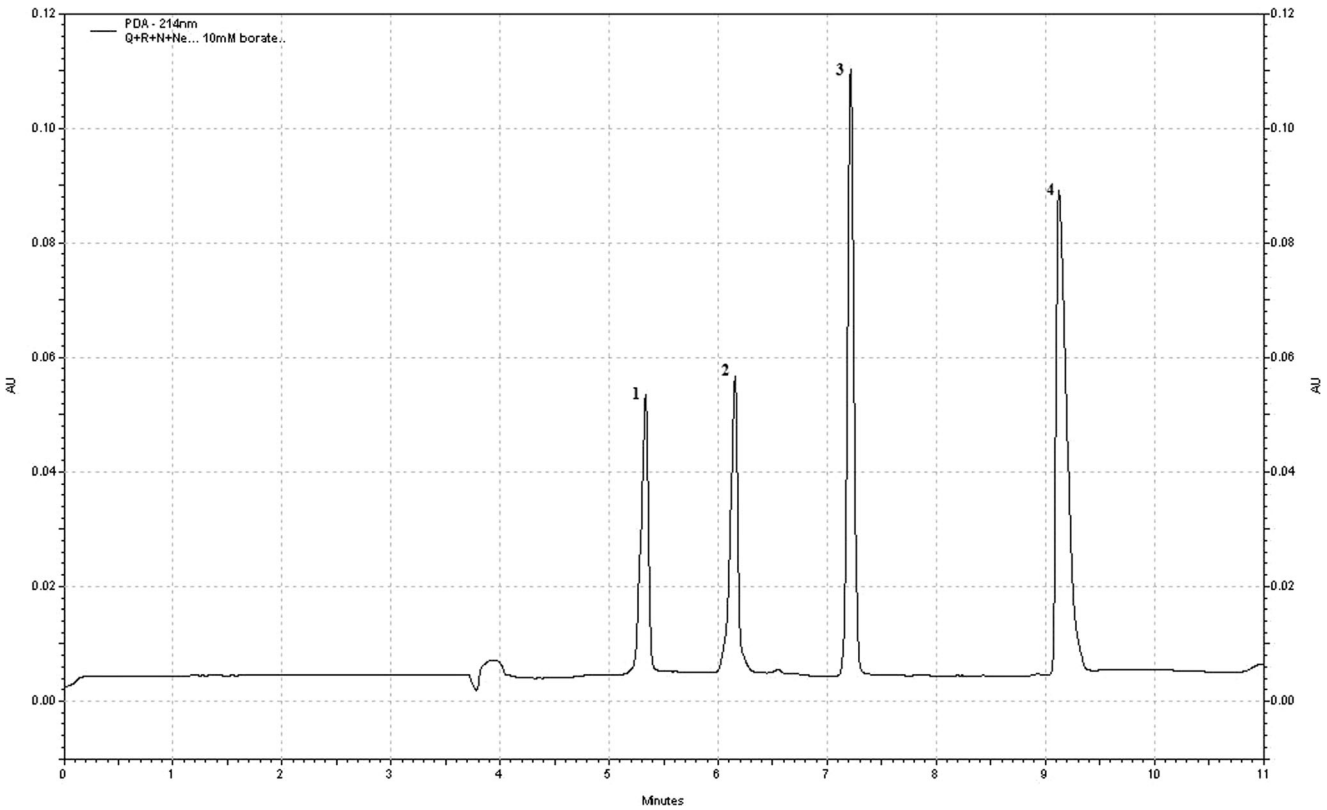

Fig. 3 Electropherogram of standard mixture solution containing naringin, rutin, naringenin, and quercetin at optimized conditions: 10 mM borate buffer, pH 8.5, 214 nm, applied voltage 25 kV. Peak identification is given in Fig. 1

Table 1 Analytical data for the determination of analytes

S. no.	Analytes	Coefficient of determination (R^2)	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Standard errors of intercept	Standard errors of slope	Intra-day precision %RSD (n = 5)		Inter-day precision %RSD (n = 3)	
								Migration time	Peak height	Migration time	Peak height
1.	Naringin	0.987	3.12–200	0.406	1.355	303.2	34.7	0.9	2.7	1.8	1.3
2.	Rutin	0.990	3.12–200	0.582	1.941	327.0	34.7	0.3	2.3	0.3	1.6
3.	Naringenin	0.987	3.12–200	0.313	1.046	554.7	63.5	0.7	1.1	1.9	1.1
4.	Quercetin	0.982	6.25–200	0.333	1.110	1108.1	127.0	0.2	2.7	1.8	2.7

determination (R^2) = 0.987 for naringin, 0.987 for naringenin, 0.990 for rutin, and 0.982 for quercetin. Regression equations, linear ranges, and values of coefficient of determination are shown in Table 1.

LOD and LOQ and Selectivity

The limit of detection (LOD) and limit of quantification (LOQ) measured as signal to noise ratio of 3:1 and 10:1 were obtained within 0.3–0.6 and 1.0–1.9 μg/mL, respectively. Table 1 shows the LOD and LOQ data of each analyte. Standard errors of intercept and slope were also calculated by Microsoft Excel data toolpak for all four analytes and values are listed in Table 1. Major possible interfering constituent of natural samples can be sugars, vitamin C, and some organic acids such as citric acid and oxalic acid. Method's selectivity in presence of all these were checked and found to have no significant interference with quantitative or qualitative determination of all four analytes.

Repeatability

The repeatability of the method was studied by performing inter- and intra-day assay by the analysis of the replicate injections of standard solution mixture. The intra-assay precision (n = 5) was performed under the same conditions, by the same analyst on the same day with the interval of 2 h, whereas the inter-assay precision (n = 3) was performed under the same conditions, by the same analyst on three consecutive days. The reproducibility of the separation in terms of migration time and peak height for all four analytes was examined and %RSD values of each are shown in Table 1.

Application of Developed CZE Method to Different Fruits

The optimized CZE method was applied for the determination of naringin, rutin, naringenin, and quercetin in different fruit samples. The detected flavonoids in samples were identified

Table 2 Assay results of the analytes in real samples

S. no.	Sample	Naringin (μg/mL) ± SD (n = 3)	Rutin (μg/mL) ± SD (n = 3)	Naringenin (μg/mL) ± SD (n = 3)	Quercetin (μg/mL) ± SD (n = 3)
1.	Fresh apple juice	<LOD	107 ± 0.4	<LOD	79.2 ± 0.5
2.	Commercial apple juice	<LOD	<LOD	10.5 ± 0.2	25.2 ± 0.1
3.	Fresh orange juice	<LOD	<LOD	9.15 ± 0.4	13.0 ± 0.2
4.	Commercial orange juice	<LOD	<LOD	71.8 ± 0.4	10.0 ± 0.2
5.	Fresh grapefruit juice	87.3 ± 2.6	1.85 ± 0.2	112 ± 0.5	14.3 ± 0.3
		Naringin (μg/g) ± SD (n = 3)	Rutin (μg/g) ± SD (n = 3)	Naringenin (μg/g) ± SD (n = 3)	Quercetin (μg/g) ± SD (n = 3)
6.	Soofi ber (large)	<LOD	<LOD	<LOD	56.8 ± 0.4
7.	Lootri ber	7.08 ± 1.5	<LOD	<LOD	<LOD
8.	Kheerol ber	<LOD	28.5 ± 0.3	1.27 ± 0.1	15.7 ± 0.2
9.	Soofi ber (small)	<LOD	<LOD	2.59 ± 0.5	<LOD
10.	Gola Lemai ber	<LOD	<LOD	1.64 ± 0.2	<LOD

<*LOD* below LOD

Table 3 Comparison of developed method with reported methods

Analyte	BGS	Migration time	Linear range (μg/mL)	LOD (μg/mL)	Detector	Reference
Naringin, naringenin, rutin	60 mM borate, pH 9	Within 19 min	1–100	0.35, 0.16, 0.18	ED	Wu et al. 2007
Quercetin, rutin	100 mM borate, pH 9	Within 9.2 min	0.15–302, 4.57–610.52	0.068, 0.264	ED	Chen et al. 2000
Naringenin, quercetin	35 mM borax, pH 8.9	Within 12 min	150, 0.1–60	0.100, 0.060	UV	Wang and Huang 2004
Rutin, quercetin	20 mM borate + 40 mM SDS + 10 % ACN, pH 8.8	Within 9 min	5–500, 5–400	1, 0.5	ED	Li et al. 2002
Quercetin, rutin	50 mM borax, pH 9.2	Within 10.9 min	2–200, 5–100	0.09, 1.1	UV	Cao et al. 2004
Quercetin, rutin	50 mM borate + 18 % MeOH, pH 9.7	Within 29 min	100–1600, 100–2000	3.5, 4.2	PDA	Sun et al. 2003
Quercetin, rutin	60 mM borate + 120 M phosphate, pH 8.8	Within 18 min	0.43–35, 0.2–16	0.02, 0.05	ED	Xu et al. 2005a, 2005b
Quercetin, naringenin	40 mM borate + 40 mM SDS, pH 9	Within 15.3 min	6.7–200, 1.7–200	0.022, 0.023	UV	Sun et al. 2008
Naringin, naringenin, quercetin, rutin	10 mM borate, pH 8.5	Within 9.1	3.12–200, 3.12–200, 6.25–200, 3.12–200	0.406, 0.313, 0.333, 0.582	PDA	Present method

by comparing their migration time and UV spectra with those of the standards using PDA detector. Quantitation was done by external calibration plot and the results are summarized in Table 2.

All four flavonoids of interest were present in fresh grapefruit juice, which were successfully identified and quantified, whereas in Kheerol ber, only three flavonoids were detected, i.e., rutin, naringenin, and quercetin. High amounts of naringin and naringenin were detected in grapefruit juice, as naringin is the main flavonoid of grapefruit. Fresh apple juice contained high amounts of quercetin. As shown in Table 2, naringin was only determined in Lootri ber, rutin was detected in Kheerol ber, naringenin was detected in Kheerol ber, Soofi ber (small), and Gola Lemai ber species, whereas quercetin was detected in Soofi ber (large) and Kheerol ber species. In orange juice, only two flavonoids, i.e., naringenin and quercetin, were detected. These findings were in general agreement with the literature data in terms of flavonoid distribution. The developed sensitive and rapid method resolved the flavonoids within 10 min.

Quantitative data of all real samples was cross checked by already reported HPLC method (Memon et al. 2013). The results obtained with HPLC method were in good agreement with developed CE method with the standard deviation of ±1.2–2.0.

Comparison of Developed Method with Reported Methods

The developed method has been compared with already reported methods of these analytes on CE. As shown in Table 3, only one report is available by PDA and our method is superior to that method both in terms of analysis time and LOD. Method developed in this study has shorter analysis time, simple background system, and comparable LOD values with most of the reported methods.

Conclusion

This paper describes the successful development of a rapid and easy CZE method for the simultaneous determination of four flavonoids, i.e., naringin, naringenin, rutin, and quercetin in different fruits like apple, orange, grapefruit, and ber using PDA detector. Under optimized conditions, all four analytes could be well determined within 10 min in a 10 mM borate buffer (pH 8.5) at an applied voltage of 25 kV. The developed CZE method demonstrates high separation efficiency and sensitivity, short analysis time, satisfactory repeatability with low operating cost, and minimal sample preparation and volume requirement.

Compliance with Ethical Standards

Funding The authors are highly thankful to the Higher Education Commission of Pakistan for providing funds under Indigenous Ph.D. fellowships for 5000 scholars (Phase-II).

Conflict of Interest Almas F. Memon declares that she has no conflict of interest. Amber R. Solangi declares that she has no conflict of interest. Saima Q. Memon declares that she has no conflict of interest. Arfana Mallah declares that she has no conflict of interest. Najma Memon declares that she has no conflict of interest. Ayaz A. Memon declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Informed consent was not applicable.

References

Akiyama T, Yamada T, Maitani T (2000) Analysis of enzymatically glucosylated flavonoids by capillary electrophoresis. J Chromatogr A 895:279–283

Awad MA, Jager AD, Westing LMV (2000) Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci Hortic 83: 249–263

Biesaga M (2011) Influence of extraction methods on stability of flavonoids. J Chromatogr A 1218:2505–2512

Bo T, Li K, Liu H (2002) Fast determination of flavonoids in Glycyrrhizae radix by capillary zone electrophoresis. Anal Chim Acta 458:345–354

Cao YH, Wang Y, Yuan Q (2004) Analysis of flavonoids and phenolic acid in propolis by capillary electrophoresis. Chromatographia 59: 135–140

Chen G, Zhang H, Ye J (2000) Determination of rutin and quercetin in plants by capillary electrophoresis with electrochemical detection. Anal Chim Acta 423:69–76

Deng F, Zito SW (2003) Development and validation of a gas chromatographic-mass spectrometric method for simultaneous identification and quantification of marker compounds including bilobalide, ginkgolides and flavonoids in *Ginkgo biloba* L. extract and pharmaceutical preparations. J Chromatogr A 986:121–127

Erlund I (2004) Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res 24:851–874

Fang T, Wang Y, Ma Y, We S, Bai Y, Zhao P (2006) A rapid LC/MS/MS quantitation assay for naringin and its two metabolites in rat plasma. J Pharm Biomed 40:454–459

Fiameogos YC, Nanos CG, Vervoort J, Stalikas CD (2004) Analytical procedure for the in-vial derivatization-extraction of phenolic acids and flavonoids in methanolic and aqueous plant extracts followed by gas chromatography with mass-selective detection. J Chromatogr A 1041:11–18

Ganzera M, Egger C, Zidorn C, Stuppner H (2008) Quantitative analysis of flavonoids and phenolic acids *Arnica montana* L. by micellar electrokinetic capillary chromatography. Anal Chim Acta 614:196–200

Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C (2007) Flavonoid composition of citrus juices. Molecules 12:1641–1673

Gil ES, Couto RO (2013) Flavonoid electrochemistry: a review on the electroanalytical applications. Braz J Pharmacog 23:542–558

Ignat I, Volf I, Popa VI (2011) A critical review of methods for characterization of polyphenolic compounds. Food Chem 126:1821–1835

Ishii K, Furuta T, Kasuya Y (2003) High performance liquid chromatographic determination of quercetin in human plasma and urine utilizing solid-phase extraction and ultraviolet detection. J Chromatogr B 794:49–56

Li X, Zhang Y, Yuan Z (2002) Separation and determination of rutin and quercetin in the flowers of *Sophora japonica* L. by capillary electrophoresis with electrochemical detection. Chromatographia 55: 243–246

Lin LZ, He XG, Lindenmaier M, Yang J, Cleary M, Qu SX, Cordell GA (2000) LC-ESI-MS study of the flavonoid glycoside malonates of red clover (Trifoliumpretense). J Agric Food Chem 48:354–365

Liu JJ, Li SP, Wang YT (2006) Optimization for quantitative determination of four flavonoids in Epimedium by capillary zone electrophoresis coupled with diode array detection using central composite design. J Chromatogr A 1103:344–349

Lotito SB, Frei B (2004) The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. Free Radic Biol Med 37:251–258

Luo M, Lu H, Ma H, Zhao L, Liu X, Jiang S (2007) Separation and determination of flavonoids in Lamiophlomis rotata by capillary electrophoresis using borate as electrolyte. J Pharm Biomed 44: 881–886

Males Z, Medic SM (2001) Optimization of TLC analysis of flavonoids and phenolic acids of Helleborus atrorubens Waldst. et Kit. J Pharm Biomed 24:353–359

Memon AA, Memon N, Luthira DL, Pitafi AA, Bhanger MI (2012) Phenolic compounds and seed oil composition of Ziziphus mauritiana L. fruit. Polish J. Food Nutr Sci 62:15–21

Memon AA, Memon N, Bhanger MI, Luthria DL (2013) Assay of phenolic compounds from four species of ber (Ziziphus mauritiana L.) fruits: comparison of three base hydrolysis procedures for quantification of total phenolic acids. Food Chem 139:496–502

Memon AF, Solangi AR, Memon S, Mallah A, Memon N (2015) MEKC method for naringenin from natural and biological samples. Anal Methods 7:4521–4527

Merken HM, Beecher GR (2000) Measurement of food flavonoids by high-performance liquid chromatography. J Agric Food Chem 48: 577–599

Pancorbo AC, Cruces-Blanco C, Carretero AS, Gutiearrez AF (2004) Sensitive determination of phenolic acids in extra virgin olive oil by capillary zone electrophoresis. J Agric Food Chem 52:6687–6693

Pascual TS, Treutter D, Rivas GJC, Santos BC (1998) Analysis of flavonols in beverages by high-performance liquid chromatography with chemical reaction detection. J Agric Food Chem 46:4209–4213

Ren ZY, Zhang Y, Shi VP (2009) Simultaneous determination of nine flavonoids in Anaphalis margaritacea by capillary zone electrophoresis. Talanta 78:959–963

Sanchez RF, Jauregui O, Lamuela RRM, Bastida J, Viladomat F, Codina C (2003) Identification of phenolic compounds in artichoke waste by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 1008:57–72

Sun Y, Guo T, Sui Y, Li F (2003) Quantitative determination of rutin, quercetin, and adenosine in Flos carthami by capillary electrophoresis. J Sep Sci 26:1203–1206

Sun Y, Fang N, Chen DDY, Donkor KK (2008) Determination of potentially anti-carcinogenic flavonoids in wines by micellar electrokinetic chromatography. Food Chem 106:415–420

Vogel H, Gonzalez M, Faini F, Razmilic I, Rodriguez J, Martin JS, Urbina F (2005) Antioxidant properties and TLC characterization of four Chilean Haplopappus species known as bailahuen. J Ethnopharmacol 97:97–100

Volikakis GJ, Efstathiou CE (2005) Fast screening of total flavonols in wines, tea-infusions and tomato juice by flow injection/adsorptive stripping voltammetry. Anal ChimActa 551:124–131

Wang SP, Huang KJ (2004) Determination of flavonoids by high-performance liquid chromatography and capillary electrophoresis. J Chromatogr A 1032:273–279

Wu W, Yan C, Li L, Liu Z, Liu S (2004) Studies on the flavones using liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr A 1047:213–220

Wu T, Guan Y, Ye J (2007) Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection. Food Chem 100:1573–1579

Xu X, Qi X, Wang W, Chen G (2005a) Separation and determination of flavonoids in Agrimonia pilosa Ledeb by capillary electrophoresis with electrochemical detection. J Sep Sci 28:647–652

Xu X, Ye H, Wang W, Chen G (2005b) An improved method for the quantitation of flavonoids in Herba Leonuri by capillary electrophoresis. J Agric Food Chem 53:5853–5857

Yu LS, Xu XQ, Huang L, Ling JM, Chen GN (2008) Separation and determination of flavonoids using microemulsion EKC with electrochemical detection. Electrophoresis 29: 726–733

Zhang S, Dong S, Chi L, He P, Wang Q, Fang Y (2008) Simultaneous determination of flavonoids in chrysanthemum by capillary zone electrophoresis with running buffer modifiers. Talanta 76:780–784

Zhang YY, Li ZC, Zhu JK, Yang ZY, Wang QJ, He PG, Fang YZ (2010) Simultaneous determination of flavonoids and anthraquinones in chrysanthemum by capillary electrophoresis with amperometry detection. Chin Chem Lett 21:1231–1234