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Micellar Electrokinetic Chromatographic Method for the Determination of Quercetin in *Psidium guajava* Leaves

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ABSTRACT: The analysis of quercetin in *Psidium guajava* leaves by micellar electrokinetic chromatography was described. The results indicated that quercetin in *Psidium guajava* leaves was mostly in the form of glycosides and the analysis of total quercetin could be performed by hydrolysis of these glycosides. The method was shown to have good accuracy and repeatability and was successfully applied to various samples.

KEY WORDS: quercetin, *Psidium guajava* Linn., capillary electrophoresis, micellar electrokinetic chromatography

INTRODUCTION

Capillary electrophoresis (CE), a technique that evolved from conventional electrophoresis, has recently gained an increasing interest in drug analysis. This is mainly because of its high efficiencies since the separations occur in small capillaries (50-100 μm of internal diameter), and therefore heat dissipation is well facilitated. Moreover, other characteristics such as ease of operation, rapid method development, economy and environmental friendliness are benefits.

Capillary zone electrophoresis (CZE) or free solution capillary electrophoresis (FSCE), the basic mode of CE, is used for charged compounds. The separation is based on the differences in the charge-to-mass ratio which then leaves the uncharged substances unaffected.

Terabe et al. (1) had introduced micellar electrokinetic chromatography (MEKC) in 1984. This technique is different from CZE only a surfactant at a concentration higher than its critical micellar concentration (CMC), in order to form the micelles, is added to the separation buffer. Using this technique, the uncharged as well as the charged compounds can be separated. The separation principle in MEKC is the result of the differences in charge-to-mass ratios, charge interaction between the analytes and the micelles, and the partition of the analytes between the micelles and the aqueous phase. Therefore, MEKC is an "interface technique" between electrophoresis and chromatography.

Guava, *Psidium guajava* Linn. (Myrtaceae) is a native plant in Tropical Africa, India, Southeast Asia, Central and South America. Its leaves and bark have a long history of use for medicinal purposes and have still been employed nowadays, especially for the treatment of diarrhea. Besides tannin which is a constituent in guava leaves at approximately 10% and known as an astringent, quercetin is also playing an important role for anti-diarrhea (2-4). Quercetin is the most widespread of all the flavonoids which are a large group of natural compounds found in plants (5), and it naturally

exists in the form of aglycone, and/or glycosides. In guava leaves, some quercetin glycosides have been isolated and identified such as quercetin-3-O-α-L-arabinose, quercetin-3-O-β-D-glucoside, quercetin-3-O-β-D-galactoside, quercetin-3-O-β-L-rhamnoside and quercetin-3-O-gentobioside (2). But the spasmolytic activity of the guava leaf remedy is suggested to be mainly due to quercetin which is obtained from the hydrolysis of its glycosides by gastrointestinal fluid (2). To date, many chromatographic techniques have been used for the separations and purification of flavonoids (6,7). With respect to the separation and determination of quercetin and its glycosides in plants and plant extracts, several techniques have been employed, e.g. spectrophotometry (8,9), thin-layer chromatography (10-12), high-performance liquid chromatography (11,13-16) and capillary electrophoresis (15,17-19). This report describes the micellar electrokinetic chromatographic method, after hydrolysis of the sample, for the determination of total quercetin in *Psidium guajava* leaves. Accuracy and reproducibility of the method have been evaluated.

MATERIALS AND METHOD

Chemicals

Quercetin and caffeine were purchased from Aldrich Chemical Co. (U.S.A.). All other chemicals and solvents were of analytical grades. The water used throughout the experiments was purified water.

Sample Preparation

Guava leaves were collected and washed with water. After air-drying the leaves were dried at 60°C for 2 days. The dried leaves were ground in a grinder and the resulting coarse powders were kept in a well-closed container.

Approximately 1 g of ground guava leaves was accurately weighed and refluxed with 30 ml of water and 30 ml of 2M HCl for 2 hours. The extract was filtered through a cotton wool and the filtrate was shaken with 3x20 ml of ethyl acetate. The combined ethyl acetate fractions were washed with 2x20 ml of water and dried with anhydrous sodium sulphate. The ethyl acetate extract was evaporated on a water-bath until dryness. The residue was redissolved in 30 ml of 60% acetonitrile, 5.0 ml of internal standard caffeine (1 mg/ml water) were added and the volume was adjusted to 50.0 ml with 60% acetonitrile.

For the recovery study of quercetin from the sample extract, the same amount of ground leaves was refluxed with 30 ml of water for 2 hours. The extract was filtered through a cotton wool and 30 ml of 2M HCl together with 5.0 ml of standard quercetin solution (500 μg/ml 60% acetonitrile) were added to the filtrate and thoroughly mixed. The spiked quercetin filtrate was shaken with 3x20 ml of ethyl acetate and further treated as described in the sample preparation.

Micellar Electrokinetic Chromatographic Conditions

Electrokinetic chromatography was performed on P/ACE 5510 system fitted with a diode array detector (Beckman, Palo Alto, CA, U.S.A.), using a fused silica capillary of 75 μm I.D., 375 μm O.D., 57 cm long with the detection window at 7 cm from the capillary outlet. The temperature of the capillary was controlled at 25°C by a liquid coolant. Integration of the electropherograms was achieved by system gold Version 810 (Beckman, Palo Alto, CA, U.S.A.).

Separation buffer was a mixture of 20 mM sodium borate and 50 mM sodium dihydrogen phosphate in an appropriate proportion to achieve pH of 8.5. Sodium dodecyl sulphate was added to the buffer to achieve a final concentration of 50 mM. The separation buffer was filtered prior to use.

Samples were introduced by pressure for 1 second, analysed with an applied voltage of 20 kV and detected at the wavelength of 214 nm. Between runs the capillary was flushed for 3 minutes with buffer and left in equilibrium for 2 minutes.

RESULTS AND DISCUSSION

Quercetin (Figure 1) is a polyphenolic compound which possesses a weak acidic property. It is considerably insoluble in water and slightly or fairly soluble in most of the organic solvents but very soluble in ethyl acetate.

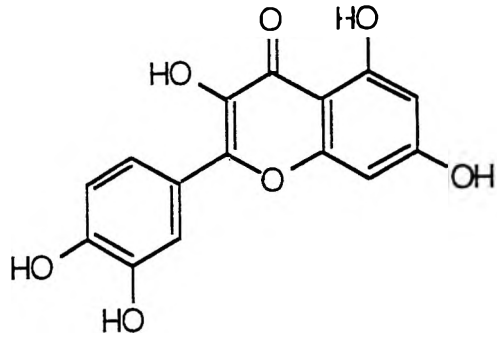

Figure 1 Chemical structure of quercetin

Capillary electrophoresis was applied for the analysis of quercetin in guava leaves due to its high efficiency for the separation and the fact that plant extracts are commonly composed of several compounds. High efficiency of the technique provides a better separation. Although quercetin can be ionized at high pH, which is feasible to be analysed in CZE mode, the peak shape of quercetin was not as good as that obtained in MEKC. In addition, the separation in MEKC is a result of several phenomenon which would provide better selectivity.

The micellar electrokinetic chromatographic method employed here was adopted from the method for the analysis of flavonoids cited from the literature (15), with some modifications. Sodium dodecyl sulphate was used as a micelle forming surfactant.

In capillary electrophoresis, an internal standard added to the sample solution prior to injection has been proven to be necessary for the area correction due to the variation of injection volumes and/or migration time shift (20). Caffeine was chosen as an internal standard due to its relative hydrophilicity compared to the sample components. Hence, the peak of internal standard was clearly separated from interference peaks as can be seen in Figures 2 and 3.

The method provided a less complex sample and quercetin was completely separated from other interferences (Figure 2) with high efficiency (plate numbers of quercetin were around 130,000). The migration time of quercetin when a sample was successively injected for 6 times was approximately 9.5 minutes with the relative standard deviation of 0.94%.

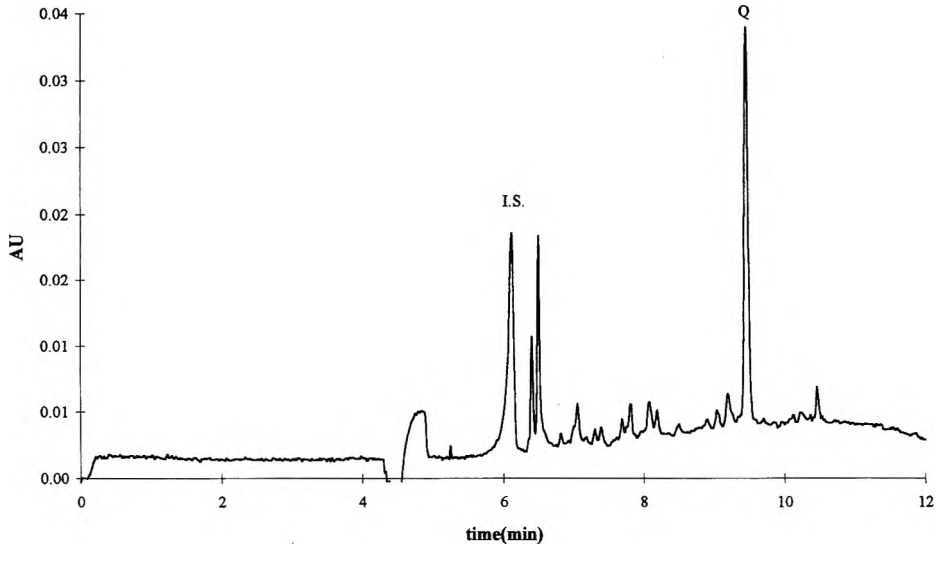

Figure 2 Typical electropherogram of guava leaf extract. Separation conditions see text, Q = quercetin, I.S. = caffeine

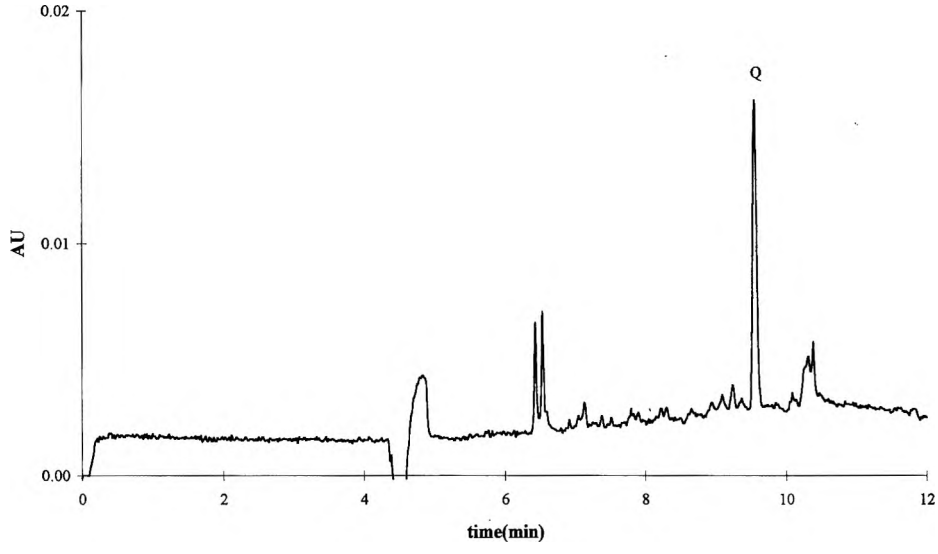

Figure 3 An electropherogram of guava leaf extract after hydrolysis (no internal standard added).

Attempt was made to replace water with ethanol (95%) in the hydrolysis. It was found that the amount of quercetin was not increased. Moreover, the electropherogram showed high background and more interferences.

Figure 3 shows the electropherogram of the sample without an internal standard after hydrolysis. Figure 4 shows the electropherogram of the sample solution when the ground leaves were refluxed with 30 ml of water for 2 hours, filtered, 30 ml of 2M HCl were added, mixed, and extracted with 3x20 ml of ethyl acetate. The sample was further treated as described in the sample preparation but the internal standard was not added prior to injection. It can be seen that no quercetin was detected and therefore indicated that most of quercetin in *Psidium guajava* leaves naturally exist in the forms of glycosides.

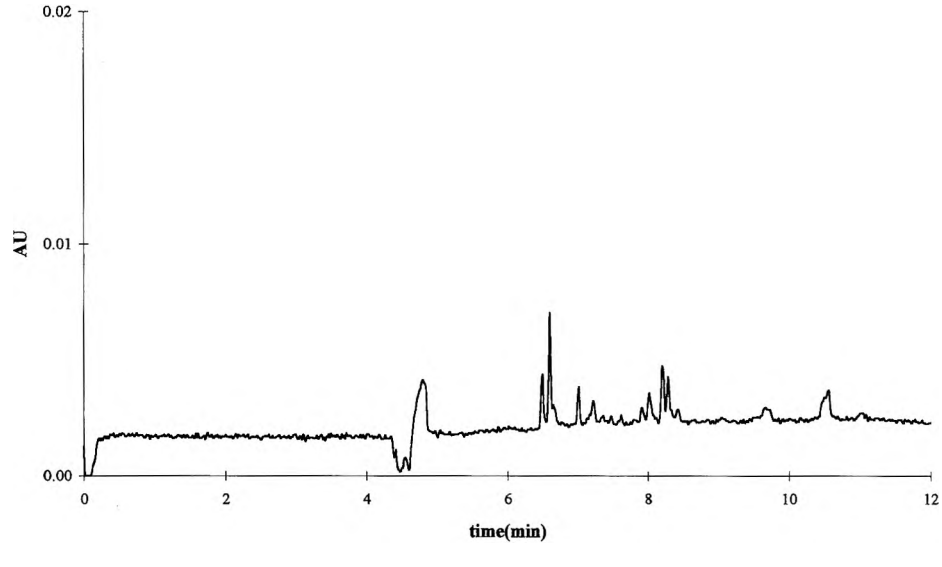

Figure 4 An electropherogram of guava leaf extract without hydrolysis (no internal standard added).

Calibration curve was constructed for quercetin in the concentration range of 20 μg-100 μg/ml using caffeine at the concentration of 100 μg/ml as internal standard. Linear regression line of the ratios of the areas of quercetin to that of the internal standard as a function of the concentrations were calculated using the least square method. Good linearity was obtained with the intercept of the regression line was not significantly different from zero.

Accuracy and Repeatability

The recoveries of quercetin spiked in sample extracts are shown in Table 1. It can be seen that spiked quercetin was completely recovered from the extracts.

Repeatability of the method was evaluated by analysing total quercetin from the ground leaves 6 times. The results are shown in Table 2.

Content of quercetin in guava leave samples

Guava leaves of 5 different sources were analysed according to the method previously described. The results are shown in Table 3.

Table 1 Recoveries of quercetin in spiked sample extracts

sample number	% found
1	103.92
2	103.59
3	101.41
4	99.02
5	98.60
6	103.26
$\bar{X}$	101.63
S.D.	2.36
R.S.D.	2.32%

Table 2 Repeatability results of the analytical method

sample number	amount found (mg/g)
1	2.973
2	3.060
3	3.148
4	3.284
5	3.012
6	2.931
$\bar{X}$	3.074
S.D.	0.135
R.S.D.	4.39

Table 3 Results of the analyses of the samples

sample number	% quercetin
1	0.23
2	0.38
3	0.31
4	0.31
5	0.31

CONCLUSION

Micellar electrokinetic chromaotography has shown to be a promising method for the analysis of quercetin in *Psidium guajava* leaves due to its high efficiency, selectivity, economy and fast analysis. Quercetin aglycone was analysed after hydrolysis of its glycosides and extracted with ethyl acetate. The recovery of extraction process for quercetin from hydrolysed samples was almost 100%. The analysis time was considerably short and quercetin was completely seperated from other interferences. The method was accurate, reproducible, applicable to raw materials of several sources and may be applied to other plants for the determination of quercetin.

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