# Determination of kaempferol and quercetin in Xindakang tablet by β-cyclodextrin modified micellar electrokinetic capillary chromatography

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**Abstract**: A method was proposed to determine kaempferol and quercetin in *Hippophae rhamnoides L* medicinal preparation xindakang tablet by β-cyclodextrin modified micellar electrokinetic capillary chromatography. Under the optimized conditions: buffer solution of 20 mmol/L $Na_2B_4O_7$-$KH_2PO_4$ (pH 9.0)-20mmol/L SDS-6mmol/L β-CD-5%(v/v) MeCN, applied voltage of 16 kV and injection time of 8s, the two analytes were separated well within 10 minutes. The calibration was linear in the 0.02-0.80 and 0.02-0.70 mg/mL range for kaempferol and quercetin, respectively. The reproducibility based on migration time and peak height were 0.47% and1.87% for kaempferol, 0.55% and 2.02% for quercetin. The detection limits based on three times noise were 0.010 mg/mL and 0.008 mg/mL for kaempferol and quercetin, respectively. The developed method was utilized to analyze real samples and running recovery experiments with satisfactory results.

**Keywords**: β-cyclodextrin (β-CD); micellar electrokinetic capillary chromatography (MEKC); flavonol; kaempferol; quercetin.

## INTRODUCTION

Favonols are one of the most important groups of flavonoids. They are very important antioxidants in biological systems and can eliminate free radicals *in vitro* very efficiently (Rice-Evans *et al.*, 1997). Flavonols containing plants are widely used in traditional Chinese medicine, the analysis of flavonols has very important medical and economic value for traditional Chinese medicine development and application. Capillary electrophoresis (CE) is a method to separate analytes under high electric field based on their differences of mass to charge ratios. It has the advantages of fast speed, high separation efficiency, low cost and have been widely used in traditional Chinese Medicine analysis (Cao *et al.*, 2010; Cheung *et al.*, 2008; Chu *et al.*, 2006; Honegr *et al.*, 2010; Lu *et al.*, 2008; Lu *et al.*, 2009;. Luo *et al.*, 2007; Ren *et al.*, 2009; Tian *et al.*, 2009; Wang *et al.*, 2003; Zhang *et al.*, 2006; Zhang *et al.*, 2011; Zhu *et al.*, 2007). Xindakang tablet is the extract of traditional Chinese medicine *Hippophae rhamnoides L* and has the functions of benefiting the heart energy, reducing blood stasis and invigorating blood circulation, diminishing inflammation and activating the spleen, etc. Kaempferol and quercetin are two acive flavonol constituents (structures are shown in fig. 1) in *Hippophae rhamnoides* L (Gao *et al.*, 1998), accurate determination of them in Hippophae rhamnoides L and its medicinal preparation is very important. Micellar electrokinetic capillary chromatography (MEKC) is a kind of separation mode of CE and it can separate both charged and neutral analytes. In this study, a method was developed to determine kaempferol and quercetin in xindakang tablet with β-cyclodextrin (β-CD) modified MEKC.

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## EXPERIMENTAL

### *Reagents and Materials*

Kaempferol and quercetin were from Sigma (St. Louis, MO, USA), Xindakang tablet was purchased from local drugstore. β-cyclodextrin was purchased from the Development Center of Special Chemical Reagents in North China. Sodium dodecyl sulfate (SDS) was from Beijing Chemical Plant. All aqueous solutions were prepared in doubly distilled water. Other chemicals were of analytical reagent grade.

### *Apparatus and Conditions*

Capillary electrophoresis experiments were performed on a 1229 type HPCE Analytical System (Beijing Institute of New Technology and Application, Beijing, China) with a fixed wavelength UV detector for absorbance measurements at 254 nm. Fused silica capillaries purchased from Yongnian Optical Fiber Factory (Yongnian, Hebei, China) were used. The dimensions of the capillary were 40 cm×50 μm i.d. The effective length of the capillary was 27 cm. The applied voltage was 16kV. Samples were injected with the hydrostatic mode at anode for 8s (10cm). The BGE was comprised of 20 mmol/L of sodium tetraborate (pH 9.0) containing 20 mmol/L SDS, 6 mmol/L β-CD and 5.0% (*V/V*) MeCN. Capillary were equilibrated daily by rinsing with 0.1 mol/L sodium hydroxide for 10 min, doubly distilled

water for 10 min and the running buffer for 15 min. To maintain good repeatability, the capillary was flushed between each separation with 0.1mol/L sodium hydroxide, water and the background electrolyte for 1 min, 2 min and 3 min, respectively.

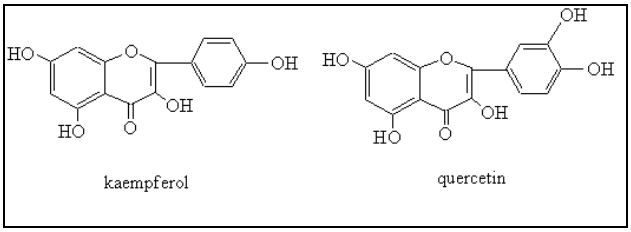


**Fig. 1**: Structures of kaempferol and quercetin

***Sample Preparation***

Xindakang tablets were pulverized after the sugar coat was taken off. Then a mass of 0.6g of the powder was accurately weighed and was put into 10 mL of methanol and soaked for 5 h. And then it was ultrasonicated for 1 h and centrifuged at a speed of 1500g for 10min. At last, the supernatant was transferred to a brown columeteric flask. The extraction was repeated three times and the extracts was combined and diluted to 50 mL. Before analysis, the sample solutions were filtered through a 0.45μm membrane filter.

## RESULTS

***Analytical Performance***

Under the optimized conditions, the separation of kaempferol and quercetin was shown in fig. 6 (a). The sample-to-sample time is less than 10 min. The detection limits and calibration parameters are summarized in table 1. Calibrations were linear in the concentration range of 0.02-0.80 and 0.02-0.70mg/mL for kaempferol and quercetin, respectively. The detection limits based on three times the noise were 0.010mg/mL and 0.008 mg/mL for kaempferol and quercetin, respectively. The reproducibility evaluated by consecutive injection of a standard solution containing 0.10 mg/mL kaempferol and quercetin 5 times. The variation in migration time and peak height were 0.47%, 0.55% and 1.87%, 2.02% for kaempferol and quercetin, respectively.

***Sample Analysis and Recovery***

The developed method was applied to determine kaempferol and quercetin in the xindakang tablet methanol extract. The electropherogram of the extract were shown in fig. 6 (b). The recovery of the method was determined with the addition of the standards (comparable to 2.00 mg/g analytes in the solid sample) in the real sample solution. The results of sample analysis and recovery were shown in table 2. The recoveries are 98.8% and 99.3% for kaempferol and quercetin, respectively.

## DISCUSSION

***Effect of buffer pH and buffer concentration***

Buffer pH can affect the electroosmotic flow (EOF) and the ioninzation of the analytes to affect the electrophoretic mobility of the analytes and their separation. The effect of buffer pH on the separation was investigated in the pH 8.0-9.6 range and the results were shown in fig. 2(a). As the buffer pH increased, the migration order between kaempferol and quercetin has changed after pH 8.2. The resolution between kaempferol and quercetin first decreased then improved with increase of pH after pH8.2. It can be seen that the two analytes can be well separated in the pH 9.0-9.2 range. As flavonols tend to be degradated at higher pH, pH 9.0 was selected.

The effects of buffer concentration on separation was investigated in the 10~30mmol/L concentration range. As shown in fig. 2 (b), the migration time becomes longer and the resolution improves with buffer concentration increase. Kaemperol and quercetin can be well separated when buffer concentration is higher than 15 mmol/L. But when buffer concentration is higher than 20 mmol/L, the baseline noise became apparent due to pronounced joule heating effect. So 20 mmol/L was selected as a compromise.

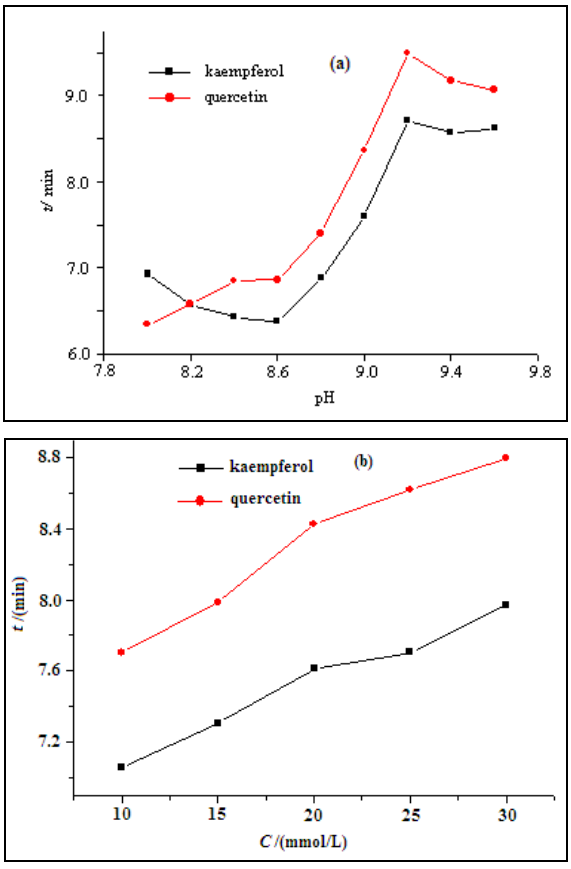


**Fig. 2**: Effects of buffer pH (a) and buffer concentration (b) on the migration of the analytes.

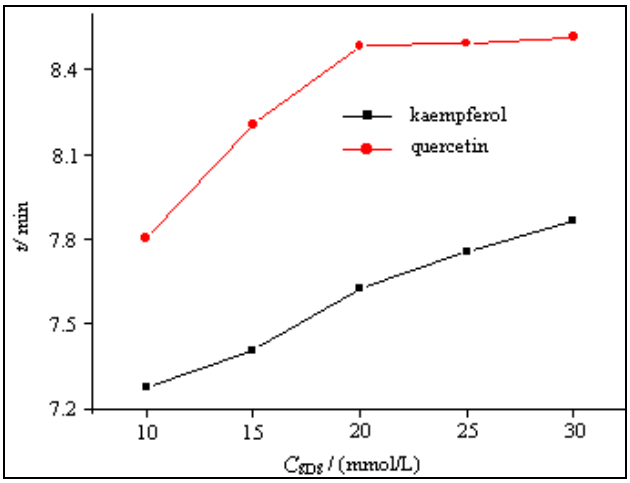


**Fig. 3**: Effects of SDS on the migration of the analytes

***Effect of SDS concentration***

*The effect of SDS concentration was studied in the 10-30mmol/L range and the results has been shown in fig. 3. The migration time of kaempferol and quercetin increaseed with SDS concentration increase and their best resolution were achieved when the SDS concentration is 20 mmol/L. So 20 mmol/L SDS was used thereafter.*

***Effect of β-CD concentration***

To further improve the separation, β-CD was incorporated into the running buffer (Deng *et al.*, 1995). The effects of β-CD was investigated in the 0-8 mmol/L concentration range. As shown in fig. 4 the migration time of kaempferol and quercetin decreased with the β-CD concentration increase. At the same time, the separation improved with β-CD concentration increase and the best resolution was obtained when β-CD concentration was 6 mmol/L, so 6 mmol/L β-CD was adopted.

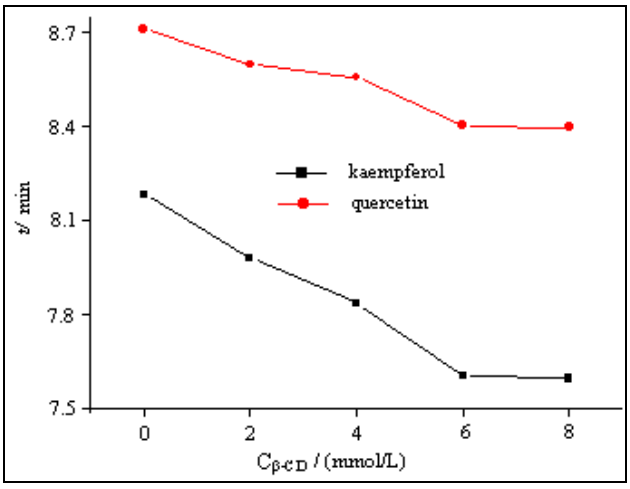


**Fig. 4**: Effects of β-CD concentration on the migration of the analyte.

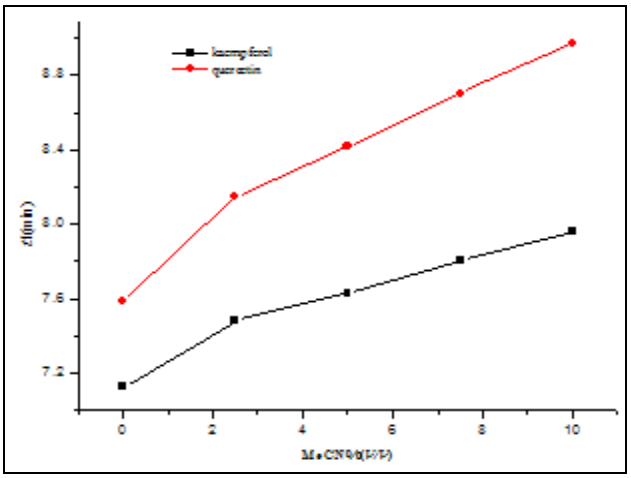


**Fig. 5**: Effects of MeCN concentration on the migration of the analytes

***Effect of organic modifier***

Organic solvents can reduce the EOF, increase the time window and improve the separation in capillary electrophoresis. In this work, MeOH, EtOH, *n*-PrOH, n-BuOH and MeCN, 5 kind of organic modifier, were tried. Based on experiments, MeCN was chosen as the optimum. The effects of MeCN was investigated in the 0-10% (*V/V*) range. The results was shown in fig. 5. As shown in fig. 5, the migration time and resolution between kaempferol and quercetin increased with MeCN concentration increase. Kaempferol and quercetin can be well separated when MeCN concentration is higher than or equal to 5% (*V/V*). In the later work, 5% (*V/V*) MeCN was adopted.

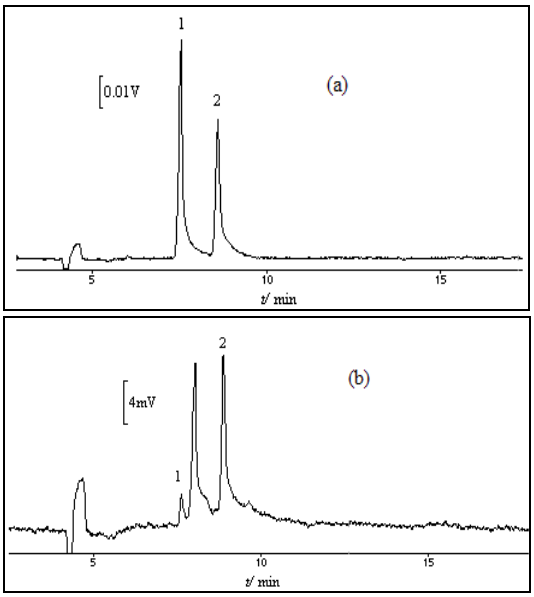


**Fig. 6**: Electropherograms of standards and the extract of xindakang tablet: 1. kempferol; 2. quercetin. Conditions: 20mmol/L sodium tetraborate (pH9.0)-20 mmol/LSDS-6 mmol/Lβ-CD-5% (*V/V*) MeCN; applied voltage: 16kV; injection time: 8s (hydrostatic: 10cm).

**Table 1**: The regression equations and detection limits[a]

| Compounds | Regression equation | Correlation coefficients | Linear range (mg/mL) | Detection limit[a]/(mg/mL) |
|---|---|---|---|---|
| kaempferol | y=92.29x+0.032 | 0.9995 | 0.02-0.80 | 0.010 |
| quercetin | y=114.34x+0.064 | 0.9983 | 0.02-0.70 | 0.008 |

Note: [a] the detection limits were based on three times the noise

**Table 2**: Results of sample analysis and the recovery (n=5).

| Compounds | Original (mg/g) | Added (mg/g) | Found (mg/g) | Recovery (%) |
|---|---|---|---|---|
| Kaempferol | 2.54 | 2.00 | 4.51 | 98.8 |
| Quercetin | 10.30 | 2.00 | 12.28 | 99.3 |

## CONCLUSION

After investigating the effects of buffer concentration and pH, SDS concentration, β-CD concentration and organic modifier, a method was developed to determine kaempferol and quercetin in xindakang tablet by MEKC. The developed method was rapid, accurate, reproducible and has great potential to be applied to the quality control.of traditional Chinese medicine.

## ACKNOWLEDGEMENT

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