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Determination of hydrochlorothiazide and rutin in Chinese herb medicines and human urine by capillary zone electrophoresis with amperometric detection

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Abstract

In this paper, capillary zone electrophoresis with amperometric detection (CZE-AD) was firstly applied to the simultaneous determination of rutin (RT) and hydrochlorothiazide (HCT) in compound Chinese herb medicines and human urine samples. The two analytes could be perfectly analyzed within 12 min and showed significant current responses at carbon electrode under the optimum conditions. It was found that the linear range of HCT was from 2.0×10^{-6} to 1.0×10^{-4} mol l^{-1} and that of RT was from 1.0×10^{-6} to 1.0×10^{-4} mol l^{-1}. Their sensitivity was determined by linear regression and calculated as 7.02×10^{4} and 2.17×10^{5} nA l mol^{-1}, respectively, and their detection limits were 5.0×10^{-7} and 2.0×10^{-7} mol l^{-1}, respectively (S/N = 3). Above results demonstrated that this method was of high sensitivity, good repeatability, high selectivity and could be used in metabolic kinetics studies of medicines. Satisfactory results were obtained when this method was used to simultaneously analyze the amounts of RT and HCT in one general compound Chinese herb medicine—Zhen Ju jiang Ya Pian and human urine samples.

Keywords: Rutin; Hydrochlorothiazide; Capillary zone electrophoresis; Amperometric detection

1. Introduction

As a general compound Chinese herb medicine, Zhen Ju Jiang Ya Pian is extensively used among Chinese hypertension patients for its special efficiency in high blood pressure cure and its annual sale in China is more than $8 million. Rutin (RT) and hydrochlorothiazide (HCT) are two of the highest amount of active ingredients in this medicine. RT is usually extracted from a Chinese herb named flos sophorae immaturus and performs a special effect on dilating blood vessels and improving the interpenetration of veins [1–3]. HCT is a kind of diuretics and also used to depress hypertension, because it can reduce the outer cell

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serum amount and blood volume by evacuating sodium ions [4].

The usual methods of determining RT or HCT are high performance liquid chromatography [5,6], spectrophotometry [7–9], chemiluminesence [10], voltammetry [11,12] etc. Until now in China, the official method used to assay the two active ingredients RT and HCT is UV and their determination is separate, so this method suffers from some shortcomings such as relatively low sensitivity and complicated operation [13,14]. In recent years, capillary electrophoresis is introduced into the medical analysis for its high separation efficiency, low sample consumption, short analysis time and relatively simple instrumentation. Quaglia et al. determined HCT in tablets by CE/CEC with linear UV–visible diode array detector and the linear range was 0.0224–0.108 mg ml^{-1} [15]; S. Hillaert et al. determined HCT in angiotensin-converting enzyme inhibitors by CE-UV, and the linear range was 0.01–0.20 mg ml^{-1} [16]. As above examples, CE is usually combined with UV–visible or laser-inducement-fluorescence (LIF) detectors, but the detection limit of UV–visible method is somewhat low, while the very sensitive LIF method needs pre- or post-column derivatization and the instrument is very expensive. Electrochemical detector is more sensitive than UV–visible detector and much cheaper than LIF detector, especially the amperometric detection can remove the interferences caused by electro inactive substances, so it is preferred to be used with CE in Chinese herbs analysis and in vivo biological analysis. Chen et al. analyzed RT in pueraria lobata and flos sophorae immaturus by capillary electrophoresis with amperometric detection (CE-AD), and the detection limit was 5.11×10^{-7} mol l^{-1} [17,18]. However, the method of simultaneous determination of RT and HCT, including CE-AD method, has not been reported.

Beacuse both RT and HCT are electroactive substances, amperometric detection is very suitable to their analysis for above advantages. The optimum conditions of simultaneous separation and detection of RT and HCT by capillary zone electrophoresis with amperometric detection (CZE-AD) were firstly studied in this paper. From experimental results, it was found that these two components could be perfectly separated within 12 min and the detection limit was as low as 2×10^{-7} mol l^{-1}. When simultaneously determining the contents of RT and HCT in Zhen Ju Jiang Ya Pian tablet and hypertension patient's urine samples by using this method, satisfactory results were obtained, which means that this method is an alternative method in the analysis of relative medicines. Because this method is of the high sensitivity, low sampling volume, good selectivity, quick speed and simultaneous assay, it is much practical than the common UV method in vivo biological analysis such as the metabolic kinetics studies of medicines.

2. Experimental

2.1. Apparatus

CZE-AD system was laboratory-built [19,20]. Electrophoresis was driven by a high-voltage supplier (±30 kV, Shanghai Institute of Nuclear Research, China). Separations were performed in a fused-silica capillary (Hebei Yongnian Laser-fiber factory, China) with 25 μm i.d., 360 μm o.d. and 40 cm long. Potential control and current output were employed by a BAS LC-3D amperometric detector (Bioanalytical System, West Lafayette, IN, USA). Electropherograms were recorded by a chart recorder (Model XWT-204, Shanghai Dahua Instrument Factory, China). Electrochemical experiments were carried out by a CHI 630 electrochemical analyzer (CHI Instruments, USA). A three-electrode system, which consisted of a carbon disk working electrode (∅ 300 μm), a saturated calomel reference electrode (SCE) and a platinum wire counter electrode, was used in both electrochemistry and detection experiments.

2.2. Preparation of carbon working electrodes

One side of the used carbon rod (∅ 300 μm) was firstly jointed with one thin copper rod (∅ 1 mm), then the carbon rod was inserted through one prepared glass tube with a normal side (∅ 5 mm) and a capillary side (∅ 1 mm). Finally, the two sides of the glass tube were sealed by nonconduct-

ing glue to make the carbon rod and copper rod stable. This carbon electrode could be used after at least 24 h air-dryness.

Prior to use, the surface of the carbon electrode was polished with emery paper and alumina powder, respectively, then it was sonicated in doubly distilled water for 3 min to get enough cleanness.

2.3. Reagents

All reagents were of analytical-reagent grade. HCT and RT were purchased from Shanghai Yuanju Reagent Company and Shanghai First Reagent Factory, respectively. Their stock solutions with a concentration of 1.0×10^{-3} mol l^{-1} were prepared with methanol and diluted with running buffer to needed concentrations in CZE experiments.

Zhen Ju Jiang Ya Pian was produced by Shanghai Third Chinese Medicine Factory.

Before CZE separations, all used solutions were filtered through 0.45 μm polypropylene acrodisc syringe filter and sonicated for 5 min to remove bubbles.

2.4. CZE operations

Before experiments, the three-electrode system was fixed in the corresponding holes of the electrochemical cell and the carbon disk electrode was carefully adjusted to make an effective conjunction to the off-side of the capillary by a three-dimension positioner.

Before each run in CZE experiments, the capillary was sequentially rinsed with 1.0 mol l^{-1} hydrochloric acid, doubly distilled water, 1.0 mol l^{-1} sodium hydroxide 3 min for each and running buffer till the current inside of the capillary reached stable. This was important to get a reproducible electroosmotic flow.

2.5. Sample preparation

One of the samples is a compound Chinese herb medicine of Zhen Ju Jiang Ya Pian. One tablet was carefully ground and then extracted with methanol. The filtrate was diluted with methanol to 10 ml volume and further diluted 50 times with running buffer before CE separation.

Another sample was urine taken from one hypertension patient 3 h after his administration of one tablet of Zhen Ju Jiang Ya Pian. This urine sample was directly analyzed by CZE-AD system just after being filtered through 0.45 μm polypropylene acrodisc syringe filter.

3. Results and discussion

3.1. Conditions of amperometric detection

Since there are hydroxyl groups in RT (Fig. 1a) molecules and there are amino groups in HCT molecules (Fig. 1b), RT and HCT can be oxidized at carbon electrode and produce current responses. Fig. 2(a) shows the cyclic voltammograms of HCT and RT in 25 mmol l^{-1} $Na_2B_4O_7$–50 mmol l^{-1} NaH_2PO_4 (pH 7.9) buffer solution at a scan rate of 100 mV s^{-1}. RT and HCT exhibited obvious anodic peaks in the voltage about +0.75 V, while the blank solution had not anodic peak in this

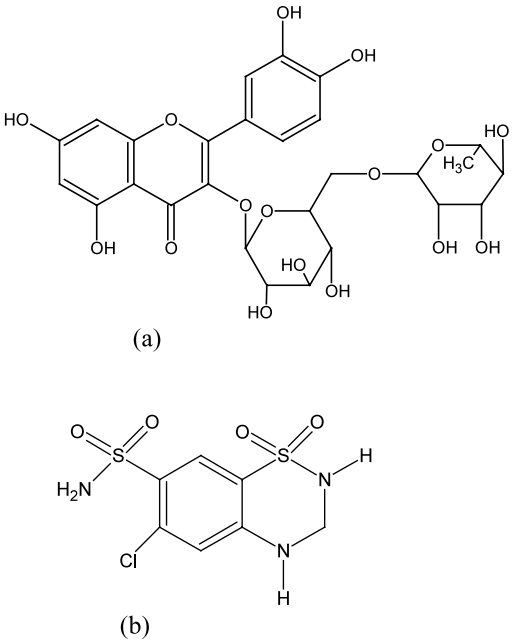

Fig. 1. Molecular structures of RT (a) and HCT (b).

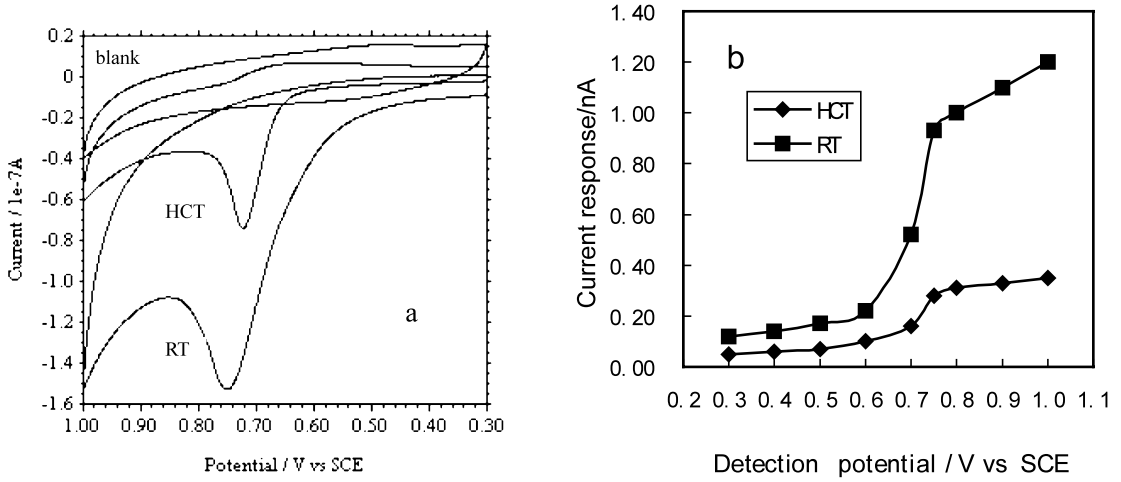

Fig. 2. (a) Cyclic voltammograms of RT and HCT with a concentration of 5.0×10^{-5} mol l^{-1} at carbon disk electrode in 25 mmol l^{-1} $Na_2B_4O_7$–50 mmol l^{-1} NaH_2PO_4 (pH 7.9) buffer solution at a scan rate of 100 mV s^{-1}. (b) HDVs of RT and HCT in CZE under different detection voltages from +0.3 to +1.0 V. Other conditions as the optimum.

voltage. Fig. 2(b) shows the hydrodynamic voltammograms (HDVs) of HCT and RT, which were obtained by monitoring their current responses after CZE separations under different applied potentials. It was found that the current responses of both analytes and the blank solution increased with the increase of the applied potential. In order to get higher sensitivity and the best signal-to-noise ratio, 0.80 V was selected as the detection potential in this experiment.

3.2. Separation conditions

RT and HCT are neutral under acidic conditions so that a CZE separation cannot be achieved for their very similar electrophoretic mobility. However, under basic borate conditions, not only RT molecules can form negative complex ions by combining their ortho-hydroxyls with borate ions [21,22], but HCT molecules can carry part negative charges by dissociating the hydrogen ions of sulfonamidic nitrogens as well. Because the ionization ability of RT is stronger that of HCT, they have different charge-mass ratios and could be separated for their different electrophoretic mobility under high electric field. Single borate buffer and borate-dihydrophosphate buffers were, respectively, tried in the experiment. The results showed that better separation was obtained when the mixture buffer was used, because the addition of dihydrophosphate into borate could change the electroosmosis flow (EOF) [23].

The effect of pH value of 25 mmol l^{-1} $Na_2B_4O_7$–50 mmol l^{-1} NaH_2PO_4 on CZE separation efficiency was studied by pH value being continuously changed from 7.5 to 10.0. The experiment results are shown in Fig. 3(a and b). It was found that with the increase of pH value, the migration time of the two analytes prolonged and their resolution enlarged, but the response current reduced obviously. Since there was a large resolution between RT and HCT, lower pH value should be selected to reduce the separation time. Under the original pH value (pH 7.9) of 25 mmol l^{-1} $Na_2B_4O_7$–50 mmol l^{-1} NaH_2PO_4, satisfactory separation efficiency and separation time were obtained.

The effect of the concentration of $Na_2B_4O_7$–NaH_2PO_4 running buffer was investigated and the results are shown in Fig. 3(c). When the total concentration of $Na_2B_4O_7$–NaH_2PO_4 running buffer was increased but their ratio was kept as 1:2, the electroosmotic flow reduced and the migration time prolonged. In this experiment, it

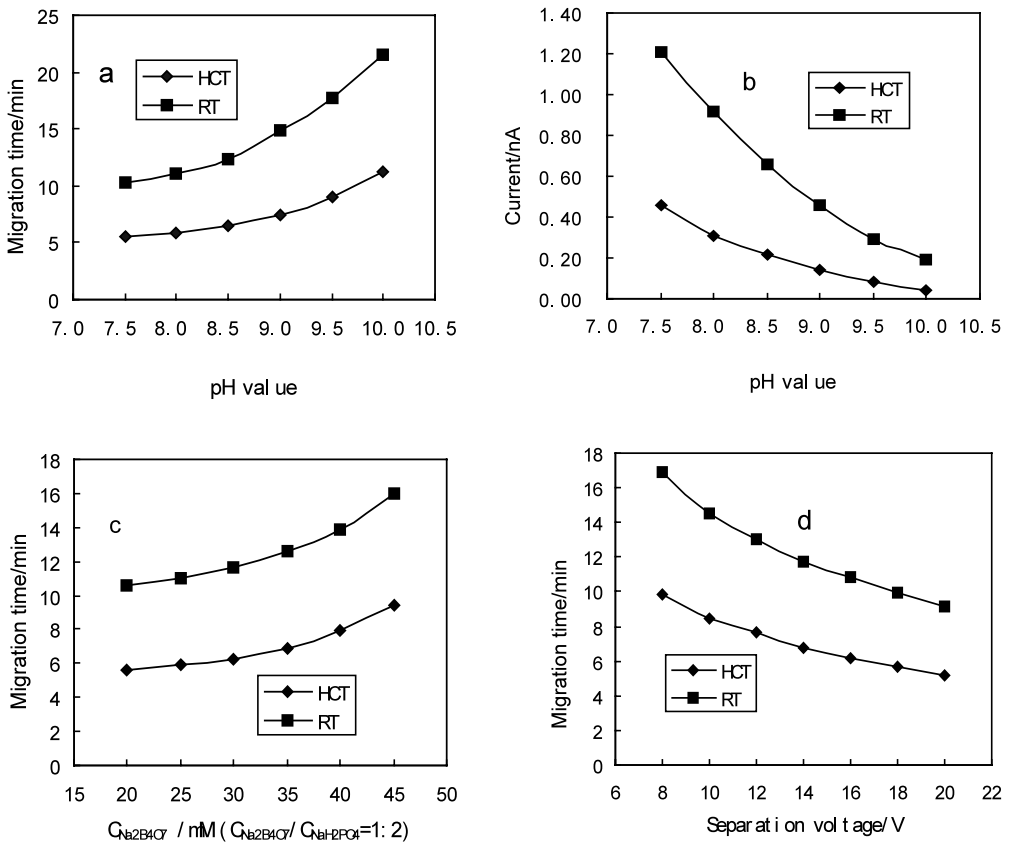

Fig. 3. Effects of pH value of running buffer on migration time (a) pH value of running buffer on current response (b) concentration of running buffer on migration time (c) and separation voltage on migration time (d).

was also found that the detection limit decreased corresponding to the reduction of current response. So, lower concentration of running buffer is suitable to reduce the separation time and improve sensitivity. In this experiment, 25 mmol l^{-1} $Na_2B_4O_7$–50 mmol l^{-1} NaH_2PO_4 was used as running buffer.

The separation efficiency of RT and HCT was investigated within the separation voltage range of 8–20 kV and the results are shown in Fig. 3(d). The migration time of the analytes was significantly shortened and their corresponding current peaks were sharpened when the separation voltage was increased. However, if the separation voltage was too large, more Joule heat was produced by the higher current inside of the capillary and separation efficiency was reduced. For a comprehensive thought, 17 kV was selected as the optimum separation voltage in this experiment.

Electrokinetic sampling was used in the CZE experiment. The sampling time changed from 4 to 16 s was tested with the other conditions as the optimum. It was found that when the sampling time was changed from 4 to 12 s, the peak currents were increased correspondingly. However, the current peaks of the analytes were obviously broadened if the sampling time was more than 12 s. So, 7 s was selected as sampling time in this experiment and satisfactory results were obtained under this condition.

The optimal conditions of this experiment were 17 kV as separation voltage, 25 mmol l^{-1} $Na_2B_4O_7$–50 mmol l^{-1} NaH_2PO_4 (pH 7.9) as buffer solution, 7 s as sampling time and 0.80 V as

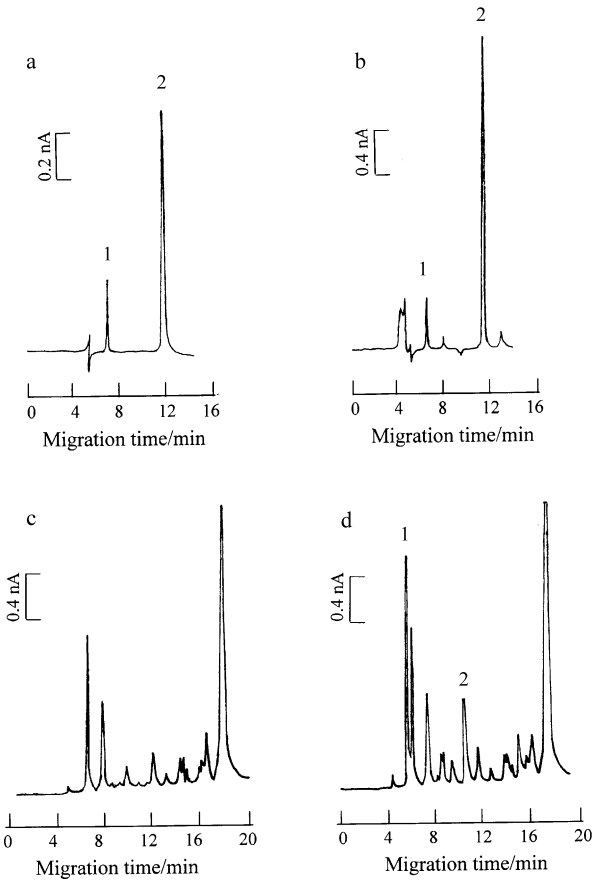

Fig. 4. Electropherograms of standard RT and HCT with a concentration of 4.0×10^{-6} mol l^{-1} (a); Zhen Ju Jiang Ya Pian (b); blank urine sample before drug administration (c); and urine sample after drug administration (d). 1-HCT, 2-RT under optimum conditions.

detection potential. Fig. 4(a) shows the electropherograms of RT and HCT with the concentration of 4.0×10^{-6} mol l^{-1} under the optimum conditions.

3.3. Linearity, repeatability and detection limits

A series of standard solutions of RT and HCT within a concentration range from 1.0×10^{-7} to 5.0×10^{-4} mol l^{-1} were analyzed under the optimum conditions and the results are shown in Table 1. The linear ranges of HCT was from 2.0×10^{-6} to 1.0×10^{-4} mol l^{-1} and that of RT was from 1.0×10^{-6} to 1.0×10^{-4} mol l^{-1}, their sensitivity was determined by linear regression and calculated as 7.02×10^{4} and 2.17×10^{5} nA l mol^{-1}, respectively, and their detection limits were 5.0×10^{-7} and 2.0×10^{-7} mol l^{-1}, respectively (S/N = 3). Above results showed that this method was very sensitive. Table 2 is the relatively standard deviations (R.S.D.) of both the migration time and peak currents of the analytes with a concentration of 5.0×10^{-6} mol l^{-1} when the analysis was repeated for six times under the same conditions. All the R.S.D.s were shown as less than 3%, which demonstrated that this method was of good repeatability.

3.4. Sample analysis

3.4.1. Analysis of Zhen Ju Jiang Ya Pian sample

This method was used to analyze HCT and RT in Zhen Ju Jiang Ya Pian according to the

Table 2
Precision of the present method ($n = 6$)[a]

Analyte	Migration time (min)		Peak height (nA)	
	Average	R.S.D. (%)	Average	R.S.D. (%)
HCT	5.9	0.96	0.31	1.89
RT	11.1	0.67	0.99	2.68

[a], The concentrations of all the two analytes were 5.0×10^{-6} mol l^{-1}.

Table 1
Regression equation[a] and detection limit[b]

Analyte	Regression equation, I (nA); C (mol l^{-1})	R (mol l^{-1})	Linear range (mol l^{-1})	Detection limit (mol l^{-1})
HCT	$I = 7.02 \times 10^{4}$ C-0.041	0.9992	2.0×10^{-6}–1.0×10^{-4}	5.0×10^{-7}
RT	$I = 2.17 \times 10^{5}$ C-0.089	0.9998	1.0×10^{-6}–1.0×10^{-4}	2.0×10^{-7}

[a], Number of calibration point was 15. [b], Detection limit was estimated according to three times of signal–noise ratio.

Table 3
Analytical results of samples ($n = 6$)

Sample	Component	Label claim	Found amount	R.S.D. (%)
Zhen Ju Jiang Ya Pian	HCT	5 mg per tablet	4.9 mg per tablet	2.0
	RT	20 mg per tablet	19.8 mg per tablet	2.4
Urine sample	HCT	–	2.6×10^{-5} mol l^{-1}	3.4
	RT	–	4.1×10^{-6} mol l^{-1}	3.6

Table 4
Recoveries of HCT and RT in sample analysis ($n = 4$)

Sample	Component	Added amount (mol l^{-1})	Found amount (mol l^{-1})	Recovery (%)	R.S.D. (%)
Zhen Ju Jiang Ya Pian	HCT	5.0×10^{-6}	4.9×10^{-6}	98.0	2.1
	RT	5.0×10^{-6}	4.9×10^{-6}	98.0	1.9
Urine sample	HCT	5.0×10^{-6}	4.8×10^{-6}	96.0	3.2
	RT	5.0×10^{-6}	4.7×10^{-6}	94.0	3.8

procedure in Section 2. The electropherograms of this medicine are shown in Fig. 4(b) and the analytical results are shown in Table 3. The found amounts of HCT and RT by this method were 4.9 and 19.8 mg per tablet and the R.S.D. were less than 2.4%. To evaluate the reliability of this method, official method UV for the assay of HCT and RT was performed. The amounts of HCT and RT obtained by UV were 4.9 and 19.7 mg per tablet and the R.S.D. were 2.6 and 2.1%, respectively ($n = 6$), so the introduced method was not only reliable but employed some advantages such as simultaneous analysis and higher sensitivity as well.

3.4.2. *Analysis of urine sample*

The urine sample was analyzed according to the procedure in Section 2.5. The electropherograms of blank urine sample before drug administration are shown in Fig. 4(c), the electropherograms of urine sample after drug administration are shown as Fig. 4(d) and the analytical results are shown in Table 3. The results show that this method can be used to study the metabolic kinetics of this medicine in human bodies without any pre-gathering.

Recovery experiments were performed four times by adding definite amounts of RT and HCT into Zhen Ju Jiang Ya Pian tablet and urine sample, respectively. The results are listed in Table 4 and show that the recoveries of both HCT and RT were ranged from 94 to 99%. It meant that this method was accurate and practical for the analysis of HCT and RT in Chinese herb medicines and human urine samples.

4. Conclusion

The experimental results demonstrated that CZE-AD was a practical method for simultaneous determination of RT and HCT in compound Chinese medicines and urine samples. This method exhibited many merits such as quickness, low sampling volume, high sensitivity and high reproducibility. Especially compared with the common UV method, this method is more sensitive, performs simultaneous assay, and could be successfully applied to the metabolic kinetics studies of medicines without any pre-gathering operation.

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