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Multiwalled carbon nanotubes-dispersive solid-phase extraction followed by high performance capillary electrophoresis for simultaneous determination of six adulterants in antihypertensive functional foods

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Abstract

A novel, simple and accurate high performance capillary electrophoresis after multiwalled carbon nanotubes-dispersive solid-phase extraction was developed for simultaneous determination of hydrochlorothiazide (HCT), chlortalidone (CTD), indapamide (IDP), reserpine (RSP), nifedipine (NDP) and valsartan (VST) in antihypertensive functional foods. After the analytes were ultrasonically extracted with acetonitrile, they were adsorbed on multiwalled carbon nanotubes (MWCNTs). Then the MWCNTs were separated through centrifugation and the analytes on the MWCNTs were desorbed with methanol. The eluent was removed through rotary evaporation and the residue was dissolved with acetonitrile-water (50:50, v/v) for CE analysis. The electrophoresis separation was carried out on an uncoated fused-silica capillary (57.0 cm total length and 50.0 cm effective length, 75.0 μm i.d.) by applying a voltage of 30 kV and the running buffer consisting of 10 mM borax buffer, 20 mM SDS and 30% acetonitrile (pH 9.7) with PDA detection at 220 nm. The capillary column temperature was set at 30 ℃. The method showed good linearity in the ranges of 1 - 50 μg/mL with LODs of 0.058 - 0.157 μg/mL. The proposed method was successfully applied for the analysis of antihypertensive functional foods with different matrix. Reserpine was detected in a sample with the content of 55.1 ± 0.9 μg/mL while other chemicals were not detected in all samples. The results of the proposed method were compared with those obtained by HPLC and there were no significant differences in the performance of the methods regarding accuracy and precision.

Keywords: Antihypertensive drugs / Micellar electrokinetic capillary electophoresis / Functional food / Multiwalled carbon nanotubes-dispersive solid-phase extraction

1 Introduction

The efficacious ingredients in antihypertensive functional foods are mainly composed of the extracts of the traditional Chinese herbs such as pueraria lobata, eucommia ulmoides, ginko biloba and pine needles. These herbs have long been used as complementary drugs to alleviate hypertension in China. But some producers illegally added antihypertensive chemicals to declare the efficacy of their products. Antihypertensive chemicals can be divided into several types according to the pharmacological mechanisms. Diuretics including hydrochlorothiazide (HCT), chlortalidone (CTD) and indapamide (IPD) inhibit the re-absorption of sodium in the renal tubules, causing an increase in urinary excretion of sodium and a decrease of the plasma volume as well as extracellular fluid volume.[1] Nifedipine (NDP), a calcium channel blocker, disrupts the movement of Ca^{2+} through calcium channels.[2] Reserpine (RSP), an alpha-2 agonist, inhibits the uptake of norepinephrine into storage vesicles, resulting in depletion of catecholamines and serotonin from central and peripheral axon terminals.[3] Valsartan (VST), an angiotensin II receptor antagonist, lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system and competes with angiotensin II for binding to the type-1 angiotensin II receptor subtype and prevents the blood pressure increasing effects of angiotensin II.[4] Intaking these antihypertensive chemicals without doctor's guidance would result in serious consequences, such as reduction of the total blood volume, leading to the risk of hypotension even collapse and the increase of blood viscosity with the risk of thrombosis. [5] Therefore, it is important to develop an effective analysis method for the measurement of these chemicals in functional foods.

Several methods have been reported for the determination of one or several aforementioned antihypertensive chemicals in pharmaceutical preparation and biological fluids. These methods include high performance thin layer chromatography, [6] high-performance liquid chromatography, [7-9] ion-pair liquid chromatography, [10] liquid chromatography/mass spectrometry (LC/MS), [11-13] liquid chromatography/tandem mass spectrometry (LC/MS/MS), [14-16] gas chromatography/mass spectrometry (GC/MS), [17,18]

derivatization spectrophotometry, [19] and voltammetry. [20] Capillary electrophoresis (CE) method has been reported by several researchers for determination of one or several antihypertensive chemicals with electrochemical detection, [21,22] electrochemiluminescence detection, [22,23] laser-induced fluorescence detection, [24] violet light emitting diode-induced fluorescence detection, [25] and mass spectrometric detection. [26] To the best of our knowledge, there are no reports on the simultaneous determination of the aforementioned six antihypertensive chemicals in functional foods by CE.

CE has proven to be an alternative method to high performance liquid chromatography. Its advantages include simplicity, time-savingness and economy. The goal of this study is to develop and validate a reliable, accurate and simple CE method for the routine quality control of the most frequently used antihypertensive chemicals in functional foods. The experimental parameters including buffer composition and concentration, buffer pH, organic modifier and its concentration, applied voltage, temperature and injection time have been optimized.

Due to the complicated matrix of functional food samples, as well as the low contents of antihypertensive chemicals, an effective sample extraction and purification technique is necessary for accurate determination of the target analytes. Solid phase extraction has been playing an increasingly important role in sample pretreatment, since it possesses the inherent advantages of high extraction efficiency, as well as high selectivity, simplicity, low solvent consumption, and rapidity. In the past few years, multiwalled carbon nanotubes (MWCNTs) have drawn great attention due to their unique tubular structures as well as excellent properties such as extremely large surface area and thermal stability. Many researchers reported that MWCNTs were powerful adsorbents for organometallic compounds [27], sulfonamides [28], barbiturates [29] in food samples.

Until now, there are no reports on MWCNTs as a SPE material for clean-up of antihypertensive chemicals in functional foods. In this study, a novel method of the MWCNTs-dispersive solid-phase extraction (MWCNTs-d SPE) combined with capillary electrophoresis was established for simultaneous determination of six antihypertensive chemicals in functional foods. The factors that may affect the

MWCNTs-d SPE procedure including extraction solvent, extraction time, the mass of MWCNTs and elution solvent were optimized.

The proposed method has been applied to the determination of the six antihypertensive chemicals in functional foods samples with satisfactory results. In addition, the method has been compared with HPLC method and no significant differences were found between the two methods regarding to the accuracy and precision.

2 Experimental

2.1 Materials and reagents

HCT, CTD, IDP, RSP, NDP, and VST standards (purity >99.0%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their chemical structures and physicochemical properties were presented in Table 1. The Log Ps and pKas of the target chemicals were quoted from DrugBank and the PubChem project.

HPLC-grade acetonitrile was purchased from ROE (ROE, USA). Water used in the experiment was from a Millipore pure water system (18.2 MΩ•cm, Millipore, USA). All other chemicals were analytical reagent grade.

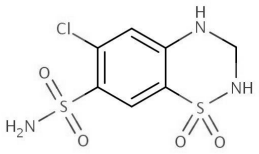
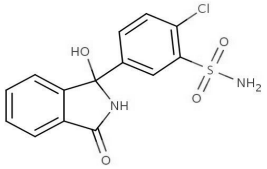
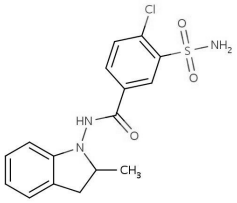
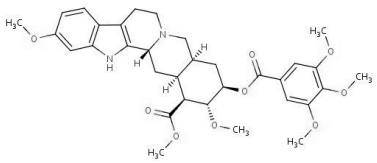
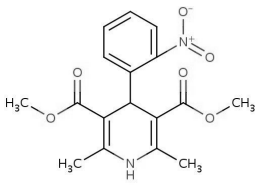
MWCNTs with average external diameters 8-15nm and purity > 95% was purchased from R&D Center of Carbon Nanotubes, Chengdu Organic Chemicals Co., Chinese Academy of Sciences. The length of the MWCNTs was 50 μm, with the specific surface area greater than 233 m^2/g. The MWCNTs were stored in a desiccator. Before use, they were dried at 100 °C for 2 h in a drying oven.

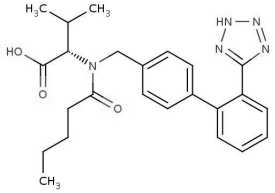
2.2 Samples

Antihypertensive functional foods including a tea substitute, a tonic wine and two capsule samples. The main ingredients of the tea substitute were ginkgol biloba and pine needles extract, pueraria and Semen Cassiae. The main ingredients of the tonic wine except alcohol (42%) and water were extract of ginkgo biloba and pueraria. The main ingredients of one capsule sample were eucommia, pueraria, cordyceps sinensis,

and polyrhachis vicina roger. The main ingredients of another capsule were sophora flower bud, chrysanthemum, flos sophorae and witloof. All of the samples were purchased from the local drug stores (Chengdu, China). For the validation of the method, fifteen simulated functional foods samples were prepared in the lab. The components of the simulated functional food samples were mainly ginko biloba extract, chitosan, medlar extract, lucid ganoderma and cordyceps sinensis extract.

Table 1 Chemical structure of the 6 analytes and their log Ps and p*K*as.

Analyte (abbreviation)	Classification	Chemical structures	Log P	p*K*a
Hydrochlorothiazide (HCT)	Diuretics		-0.07	7.9
Chlortalidone (CTD)	Diuretics		0.85	8.58
Indapamide (IDP)	Sulfa diuretic		2.2	8.8
Reserpine (RSP)	Alpha-2 agonists		3.2	6.6
Nifedipine (NDP)	Calcium channel blockers		2.2	5.33

Valsartan (VST)	Angiotensin II receptor antagonists		5.27	4.37

2.3 Instrumental conditions

The analysis was performed on a Beckman P/ACE^{TM} MDQ capillary electrophoresis system (Fullerton, CA, USA) equipped with an autosampler, a photodiode array detector (PDA), a temperature controlling system (15–60°C), and a power supply. Beckman P/ACETM station software (version 1.2) was used for the instrumental control, data acquisition, and analysis. Electrophoretic separation was performed on an uncoated fused silica capillary (57.0 cm total length, 50.0 cm effective length and 75 μm i.d., Beckman Coulter, Inc, USA). The running buffer was consisted of 10 mM borax buffer, 10 mM SDS and 30% acetonitrile (pH=9.7). The buffer was ultrasonically degassed for 15 min and centrifuged at 10,000 rpm for 10 min before use.

Before each injection, the capillary was preconditioned with the running buffer for 5 min at 20 psi pressure. Injection was carried out under pressure at 0.5 psi for 10 s. The wavelength of PDA was set at 220 nm. The capillary temperature was kept constant at 30 °C and a voltage of 30 kV was applied (positive polarity).

Other instruments involved in sample preparation including analytical balance (BS110S, Sartorius, Germany), centrifuge (TGL-16B, Anke, Shanghai, China), ultrasonic cleaner (KQ-250, Dianshanhu Instrument, Kunshan, China), and vortex mixer (Yamato MT-5, Japan).

2.4 Sample preparation

The tea subsititute sample was ground into powder, and the capsule sample was decladed then mixed well. A 2.0 gram of homogenized tea subsititute or capsule sample was weighed into a 50-mL plastic centrifuge tube, followed by adding 8 mL of acetonitrile. The sample was subsequently ultrasonically extracted for 15 min. Then the mixture was made up to 10 mL with acetonitrile and mixed well and finally centrifuged

at 5,000 rpm for 10 min. The wine sample was directly centrifuged at 5,000 rpm for 10 min.

Two milliliters of supernatant solution was pipetted into 10-mL centrifuge tube containing 6.00 mL water and 20 mg of MWCNTs. After vortex agitated for 2 min, the mixture was centrifuged at 5,000 rpm for 3 min and the solution was discarded. Two milliliter methanol-water solution (3:7, v/v) was added, then it was vortex agitated for 2 min, then centrifuged at 5,000 rpm for 3 min and the supernatant solution was discarded. Finally, to elute the target analytes from MWCNTs, 5.00 mL methanol was added, vortex agitated for 2 min, and then centrifuged at 5,000 rpm for 3 min, and 4.80 mL of supernatant was transferred into a 10-mL centrifuge tube. The eluent was dried by rotary evaporation at 50 ^{o}C and the residue was redissolved in 1000 μL acetonitrile-water (50:50, v/v) for CE analysis (the volume of acetonitrile-water could be varied according to the contents of the target chemicals).

2.5 Quantification analysis

The stock solution of each standard was prepared individually in acetonitrile at 1.00 mg/mL and stored in refrigerator at 4°C. A mixed stock standard solution was prepared by suitable dilution with acetonitrile-water (50:50, v/v). Working solutions in the range of 1–50 μg/mL for all analytes were prepared by further dilution of the stock solutions with acetonitrile-water (50:50, v/v) and finally analyzed. Fig.1 presents the electropherogram of a mixed standard solution.

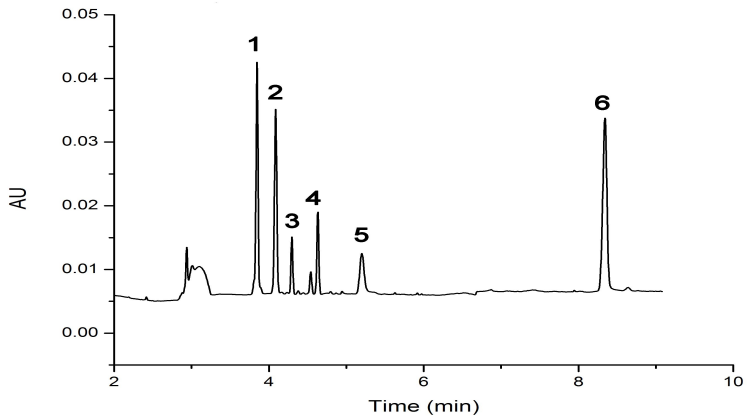


Fig. 1 The electropherogram of the mixed standard solution.
The peak identifications: RSP (3.91 min), CTD (4.11 min), IDP (4.28 min), HCT (4.67 min), NDP (5.31 min) and VST (8.60 min).

3 Results and discussion

3.1 Optimization of CE conditions

The buffer composition and its concentration, as well as its pH, are important influential factors for electrophoresis separation of the analytes. Two running buffers, *i.e.,* phosphate and borax, at different pH and molarities were tested for CE analysis and no obvious difference was found in migration time, resolution, peak shape, peak height and baseline noise. The borax buffer was selected as running buffer because it could provide better reproducibility.

3.1.1 The pH of borax buffer

The effect of the pH of the running buffer was investigated within the range of 8.0 - 9.7 with the running buffer consisting of 10 mM borax, 10 mM SDS and 30% acetonitrile. The results demonstrated that the target analytes were well separated when the pH was 9.0 to 9.7. But when the pH was 9.0, the migration time of VST was too long (16.8 min）. When the pH was 9.7, the migration time of VST would be shortened to 12.0 min, so the pH of the running buffer was adjusted to 9.7. (the supporting information Fig. S1).

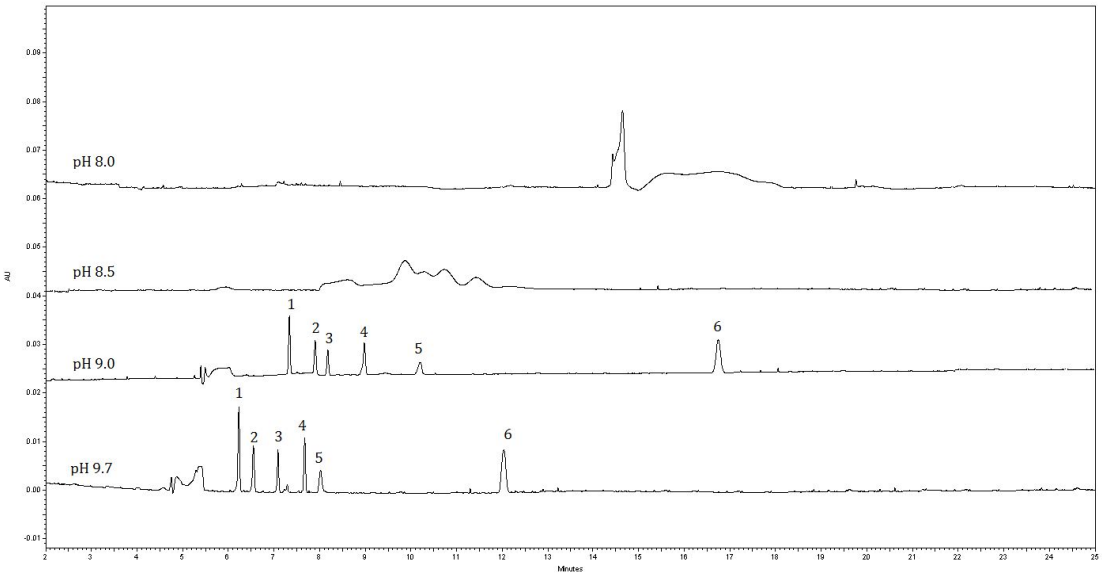


Figure S-1. Effect of borate buffer pH on separation of the studied chemicals.
Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.2 The borax buffer concentration

Buffer concentration also significantly affects the separation performance through its influence on the EOF and the current produced in the capillary. The effect of borax concentration of running buffer was examined by changing its concentration from 10 to 40 mM. The results showed that with an increase in borax concentration, migration times and current increased, yet no improvement on resolution was observed. So 10 mM concentration of borax buffer was used to reduce the analysis time, as well as to obtain better resolution and acceptable background current. (the supporting information Fig. S2).

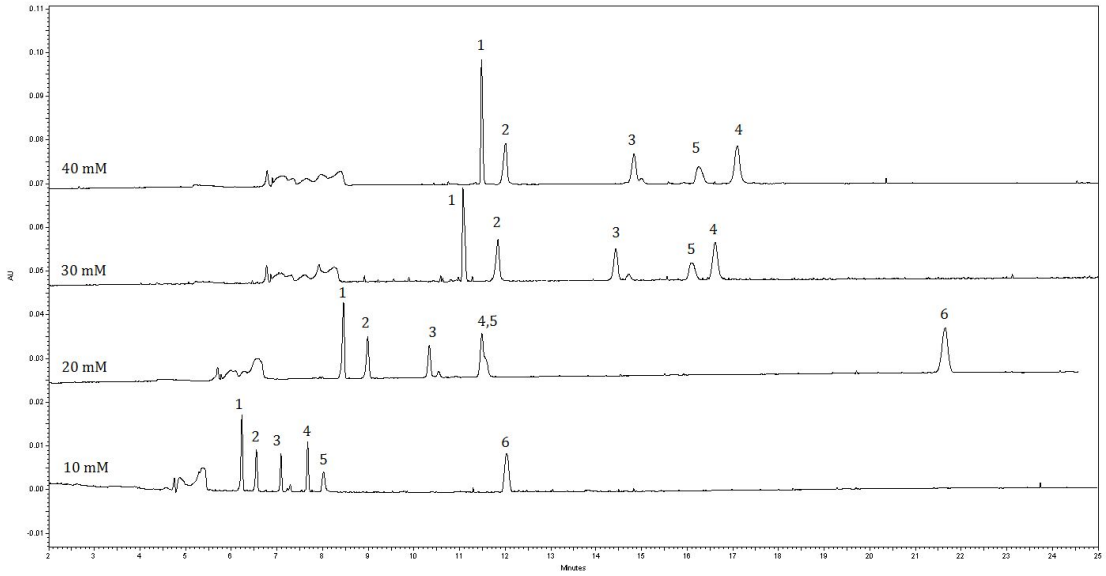


Figure S-2. Effect of borate concentration on separation of the studied chemicals. Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.3 The SDS concentration

The effect of SDS concentration in running buffer was examined by varying its concentration from 0 to 40 mM. When no SDS was added, the resolution was not satisfactory. When the suitable amount of SDS was added in the running buffer, both resolution and migration times increased. Finally, 20 mM SDS was used to reduce the analysis time and to obtain better resolution and acceptable background current. (the supporting information Fig. S3).

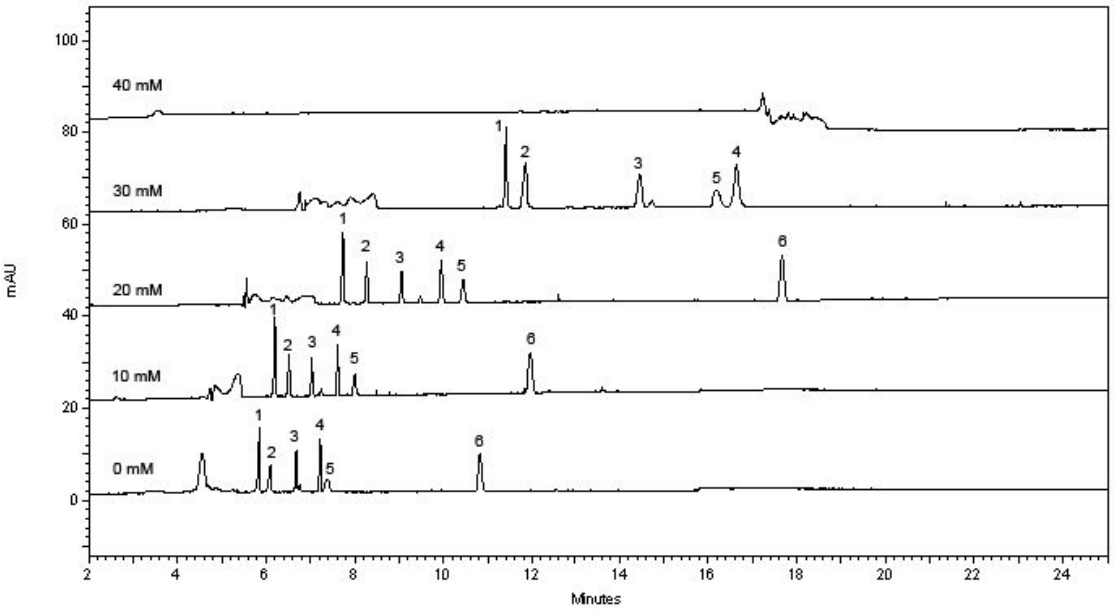


Figure S-3. Effect of SDS concentration on separation of the studied chemicals.
Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.4 The organic modifier

The addition of organic modifier into the running buffer could change its viscosity, dielectric constant, and zeta potential, so as to improve the separation and resolution of the analytes. In order to investigate the effect of organic modifier, acetonitrile was added at different percentage (0, 10, 20, 30 and 40%, v/v) into the running buffer of 10 mM borax buffer, containing 20 mM SDS at pH 9.7. The concentration of acetonitrile affected the migration time and the resolution of all of the target analytes obviously. When the percentage of acetonitrile was below 20%, six analytes could not be effectively separated. When its percentage reached to 40%, the migration time of VST was too long (>25 min). So 30% acetonitrile in running buffer was used. (the supporting information Fig. S4).

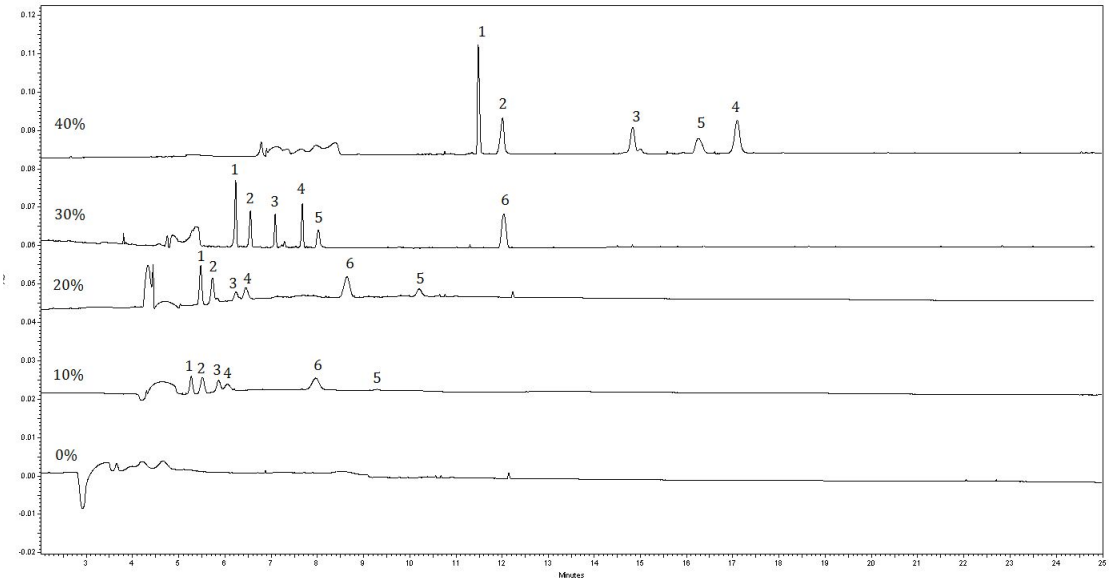


Figure S-4. Effect of acetonitrile proportion on separation of the studied chemicals. Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.5 The applied voltage

The influence of the applied voltage (15 to 30 kV) was investigated under the optimized running buffer. As expected, with the increase of the applied voltage, the EOF increased too, leading to shorter analysis times. To reduce the analysis time, the voltage 30 kV was used in the following experiment. (the supporting information Fig. S5).

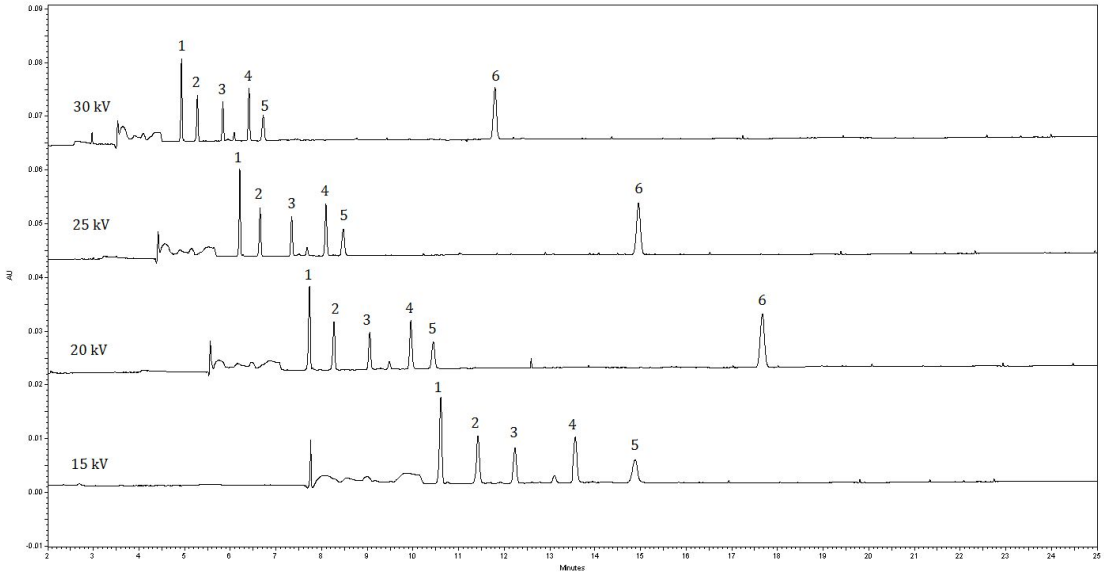


Figure S-5. Effect of applied voltage (kV) on separation of the studied chemicals. Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.6 The capillary cartridge temperature

The viscosity of the running buffer is affected by capillary temperature, so changes in temperature would change EOF, electrophoretic mobilities and injection volume. The influence of the temperature (15, 20, 25, 30, 35 and 40 ◦C) on the separation was investigated. The results showed that 30 ◦C could get the best resolution. (the supporting information Fig. S6).

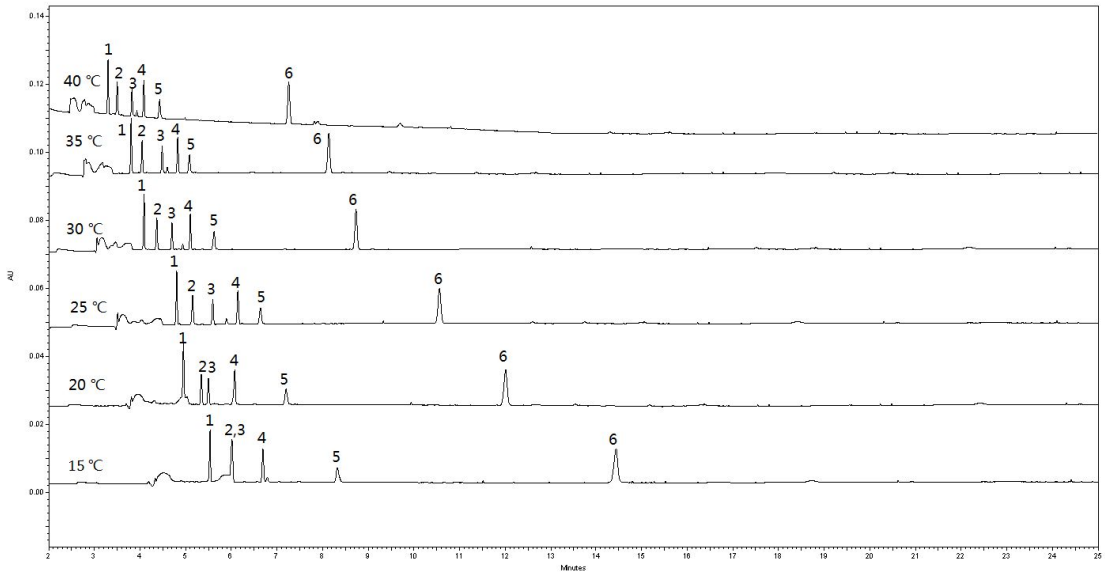


Figure S-6. Effect of capillary cartridge temperature (◦C) on separation of the studied drugs.

Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.7 The injection time

Injection time affects the peak width, peak height, as well as migration time. Sample solutions were injected at 0.5 psi while the injection time varied from 5 to 30 s. If the injection time was longer than 25 s, the peak widths of the analytes would broaden and their peak shapes would deteriorate. So the injection time used in the following experiment was 20 s. (the supporting information Fig. S7).

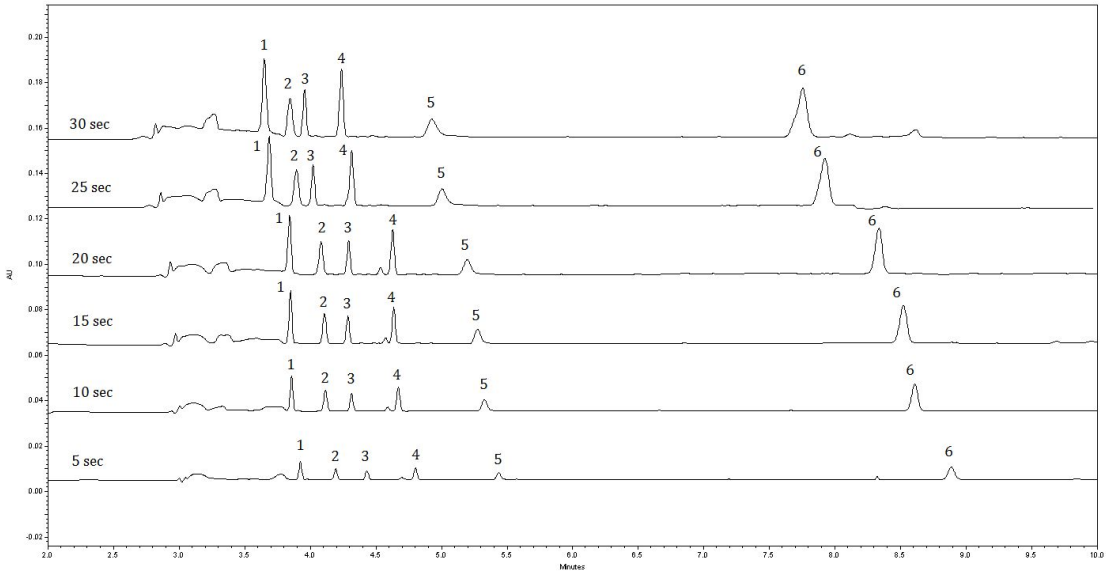


Figure S-7. Effect of injection time (sec) on separation of the studied chemicals.
Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.1.8 The detection wavelength

In order to improve the detection limits of the method, the sensitivities at different wavelength (190–300 nm) were compared. The results showed that 220 nm could give all of the six analytes satisfactory sensitivities. (the supporting information Fig. S8).

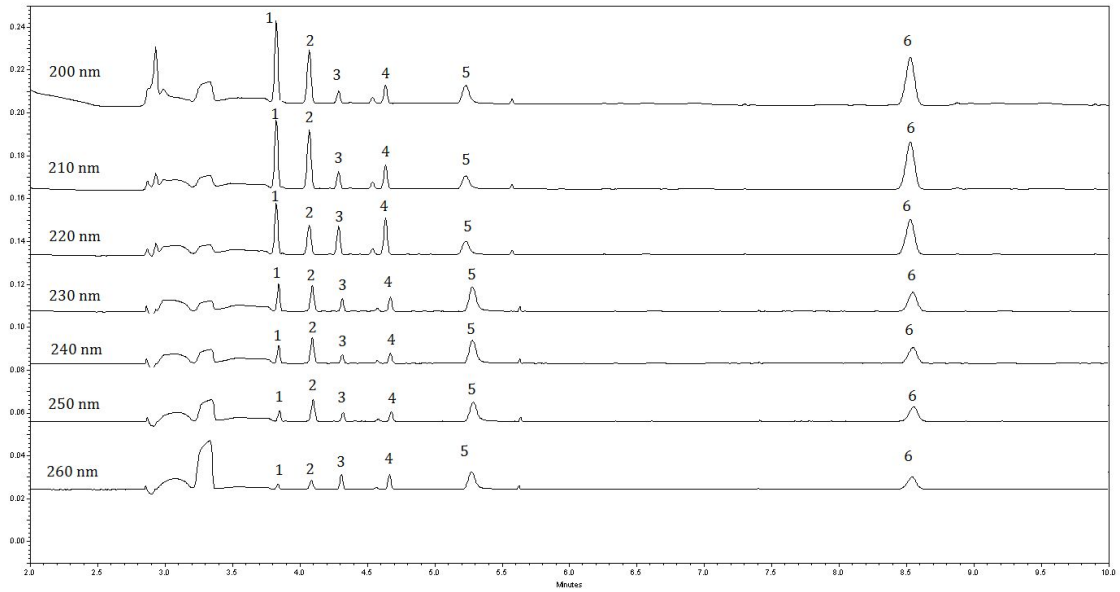


Figure S-8. Selection of the detection wavelength.
Peak indentifications: 1-RSP, 2-CTD, 3-IDP, 4-HCT, 5-NDP, 6-VST.

3.2 Optimization of ultrasonication extraction

All of the analytes are soluble in methanol, acetonitrile, and ethyl acetate. Therefore, extraction solvents including methanol, acetonitrile, methanol-acetonitrile solution (1:1, v/v), were investigated. The ultrasonication time was set at 15 min. The experimental results showed that acetonitrile could give highest extraction efficiencies (75.0 % to 96.5%) for all of the analytes. Methanol could give equivalent extraction efficiencies for all of the analytes except IDP (42.3%). Accordingly, acetonitrile was chosen as the extraction solvent throughout the experiment.

3.3 Optimization of MWCNTs-d SPE conditions

The matrix of the functional food samples were very complex and the co-existent substances would interfere with the measurement. So the sample solution should be cleaned-up before analysis. In this study, MWCNTs with average external diameters (8-15nm) was used as sorbent in MWCNT-d SPE procedure. The related experimental conditions have been carefully optimized to achieve satisfactory enrichment, clean-up performance and reasonable recoveries.

3.3.1 Optimization of adsorption procedure

Antihypertensive chemicals are medium polar compounds and MWCNTs is nonpolar substance. According to solid phase extraction principle, sample solution (acetonitrile) should be mixed up with proper amount of water before MWCNTs-d SPE procedure to increase the polarity of the sample solution and thus make target chemicals be adsorbed onto the MWCNTs. The effect of different proportions of acetonitile-water (1:9, 2:8, 3:7, 4:6, 5:5, v/v) on adsorption percentages was investigated. The experimental results showed that the adsorption percentages for the six analytes were greater than 94.6% when the proportion of acetonitrile-water was 3:7 (i.e. the water percentage was 70 %). So 2 mL of the sample solution (acetonitrile) was mixed with 6 mL of water at the loading step, i.e. the acetonitrile-water proportion was 1:3(v/v) or the water percentage was 75 % (>70%).

The effect of adsorption time on the adsorption percentage was studied for acetonitrile-water solution (1:3, v/v) without pH adjustment at different vortex time (1, 2, 3, 5 and 10 min). The spiked concentrations of the six analytes were all 1 μg/mL. The

results indicated that vortex time longer than 2 min had no significant effect on the adsorption percentages of the target chemicals. The results were in accord with the reports from Shen [30] and Hou [31], who both found that adsorption of aromatic compounds onto MWCNTs was 2 min. Accordingly, a 2 min vortex time was used for the following experiments.

The effects of the amounts of MWCNTs (5, 10, 15, 20, 40 mg) on adsorption percentages were compared at spiked 1 μg/mL in acetonitile-water solution (1:3, v/v) using 2 min vortex time. The results showed that the adsorption percentages were improved as the amount of MWCNTs increased from 5 to 20 mg for all six target chemicals. When 20 mg MWCNTs was employed, the adsorption percentages of the ten chemicals were greater than 91.4%. So 20 mg of MWCNTs was used in the following experiment.

3.3.2 Optimization of elution solvent

Five organic solvents including acetonitrile, methanol, acetone, dichloromethane and methanol-dichloromethane (1:1, v/v) were compared to obtain acceptable elution efficiencies. The results showed that among all the solvents, methanol exhibited satisfactory elution efficiencies for all the analytes (Fig. 2). Therefore, methanol was selected as the elution solvent.

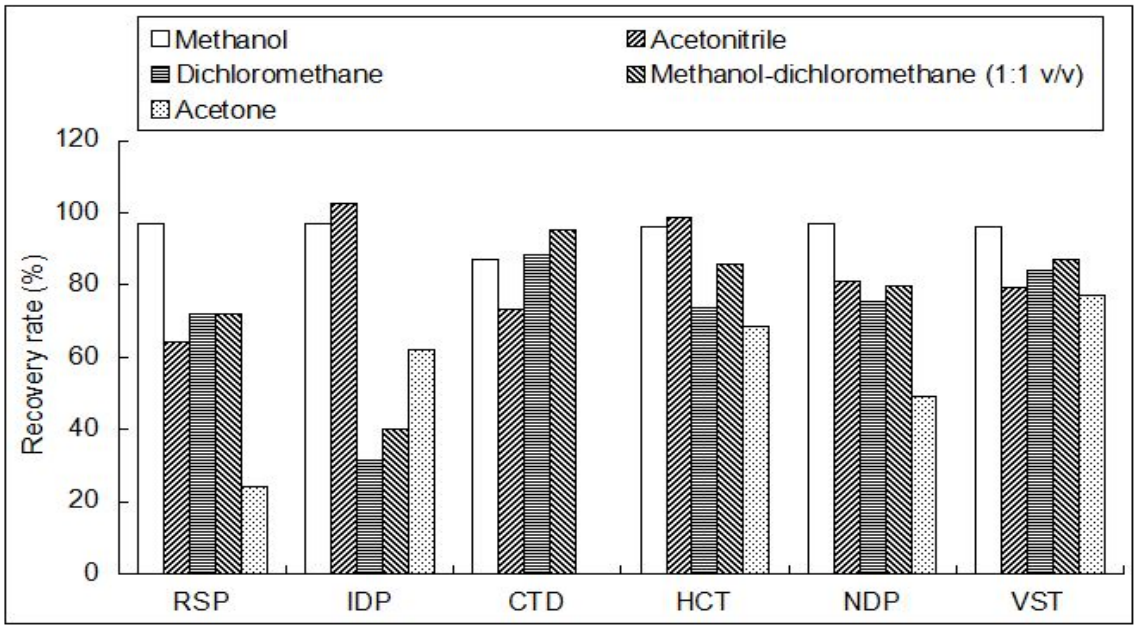


Fig. 2 The elution efficiencies of the different solvent.

The volume of eluent affected the elution performances significantly. To compare the effect of the eluent volume on the elution efficiencies, different volumes (1.0, 2.0, 3.0, 5.0 and 10.0 mL) of methanol were investigated. With the increase of volume of the eluent, the elution efficiencies increased too, but too large volume of eluent would need longer evaporation time. Therefore 5.0 mL of methanol was used for elution of all the analytes (with the elution efficiencies of 93.1% to 103.5%).

Fig. 3 presents the electropherograms of three samples with different matrix and the same sample spiked with the mixed standard solution. And it also shows the differences before and after the the MWCNTs-d SPE procedure**.** It is very obvious that MWCNTs-d SPE could effectively eliminate the co-existent interfering species.

3.4 Validation of the method

3.4.1 Linearities

The calibration curves were constructed by plotting the peak area against the analytes′ concentration (μg/mL). The linear ranges were found to be 1–50 μg/mL for the six target analytes (Table 2).

Table 2 The performances of the method.

Analyte	Migration time (min)	Linear range（μg/mL）	Calibration equation	LOD (μg/mL)	LOQ (μg/mL)	Correlation coefficient (r)
RSP	3.91	1-50	y= 10951x + 281	0.092	0.303	0.9998
CTD	4.11	1-50	y= 6472x + 134	0.157	0.518	0.9999
IDP	4.28	1-50	y= 6489x + 214	0.154	0.508	0.9999
HCT	4.67	1-50	y= 9481x + 284	0.105	0.347	0.9998
NDP	5.31	1-50	y = 9017x + 463	0.109	0.360	0.9998
VST	8.60	1-50	y = 17306x + 745	0.058	0.191	0.9997

3.4.2 LODs and LOQs

The LODs and LOQs were calculated as three times and ten times of signal-to –noise ratio (S/N=3, S/N=10), respectively. LODs and LOQs of the method were 0.058 - 0.157μg/mL and 0.191 - 0.518μg/mL, respectively (Table 2).

3.4.3 Precisions

The intraday and interday precisions of the method were assessed using 5 μg/mL of the mixed standard solution and three replicate determinations of standard solution (Table 3). From Table 3, we can see that the relative standard deviations (RSDs) of the method for migration time were 3.6% - 6.2% (intraday) and 3.9% - 5.9% (interday), respectively and RSDs for peak area were 2.2% - 4.1% (intraday) and 2.6% - 3.9% (interday), respectively.

3.4.4 Accuracies

Into fifteen simulated samples were spiked 1 mg/kg, 8 mg/kg and 20 mg/kg mixed standard solution, respectively. Table 4 summarizes the mean recoveries for all analytes in fifteen simulated samples. The average recoveries for the samples were from 73.7% to 99.5%, with the RSDs of less than 9.8%.

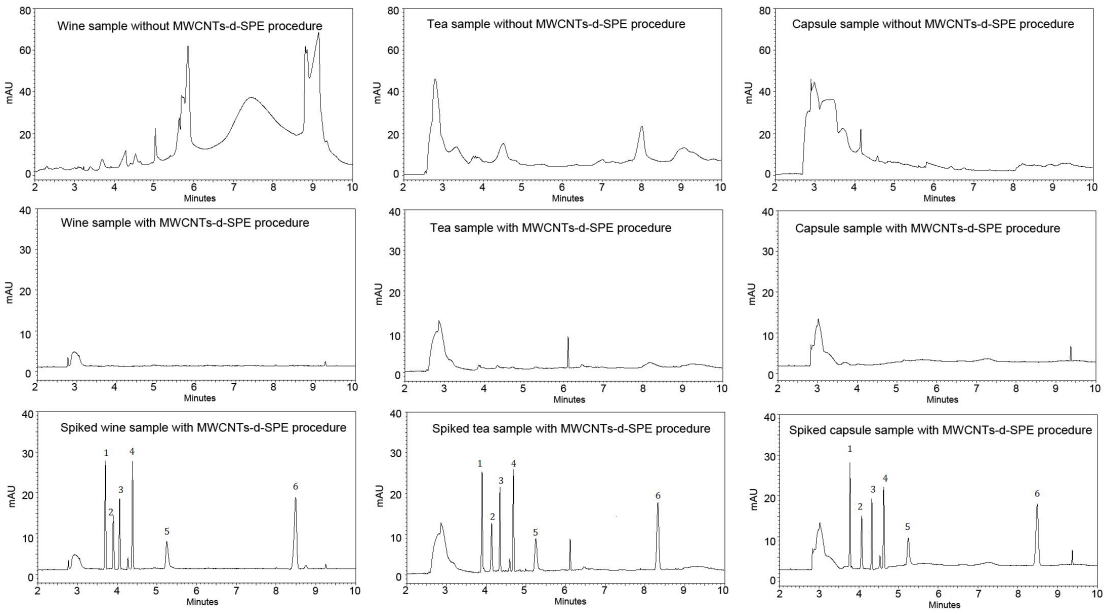


Fig. 3 The electropherograms of three samples with different matrix without MWCNTs-d-SPE procedure (above); the same samples cleaned-up with MWCNTs-d-SPE procedure (middle) and the same samples spiked with the mixed standard solution subjected to MWCNTs-d-SPE procedure (lower).
Peak indentifications: 1. RSP; 2. CTD; 3. IDP; 4. HCT; 5. NDP; 6. VST.

3.4.5 Method comparison

The proposed method was compared with our proposed HPLC method. The HPLC method used a C18 column as separation column, with the mobile phase of acetonitrile - 0.03 mM phosphatic buffer solution (pH 3.0) in gradient elution program and UV

detection at 220 nm. The results showed that the proposed method was superior to the HPLC method in respect of the total analysis time (the separation and rehabilitation time needed 34 min for HPLC method), as well as the injection volume and the analytical cost. The detection limits of the proposed method were 0.058 - 0.157μg/mL, and those of HPLC were 0.014 to 0.053 μg/mL.

Table 3 The precisions of the method.

Intraday						
Analyte	Migration time			Peak area		
	Range (min)	Mean (min)	RSD (%)	Range	Mean	RSD (%)
RSP	3.87 - 3.94	3.91	3.6	5.43×10^4 to 5.49×10^4	5.47×10^4	3.5
CTD	4.06 - 4.16	4.11	5.3	3.18×10^4 to 3.26×10^4	3.23×10^4	3.4
IDP	4.24 - 4.34	4.30	5.2	3.21×10^4 to 3.27×10^4	3.24×10^4	3.6
HCT	4.61 - 4.73	4.68	6.2	4.70×10^4 to 4.80×10^4	4.74×10^4	4.1
NDP	5.26 - 5.36	5.31	4.3	4.48×10^4 to 4.51×10^4	4.50×10^4	2.2
VST	8.55 - 8.66	8.61	5.5	8.61×10^4 to 8.70×10^4	8.65×10^4	3.3
Interday						
Analyte	Migration time			Peak area		
	Range (min)	Mean (min)	RSD (%)	Range	Mean	RSD (%)
RSP	3.81 - 3.97	3.89	5.3	5.38×10^4 to 5.45×10^4	5.41×10^4	2.6
CTD	3.97 - 4.10	4.06	5.9	3.26×10^4 to 3.30×10^4	3.28×10^4	3.8
IDP	4.18 - 4.36	4.28	4.2	3.17×10^4 to 3.23×10^4	3.21×10^4	3.6
HCT	4.58 - 4.64	4.61	4.1	4.76×10^4 to 4.79×10^4	4.78×10^4	3.2
NDP	5.20 - 5.31	5.26	3.9	4.41×10^4 to 4.49×10^4	4.46×10^4	3.9
VST	8.52 - 8.68	8.57	5.3	8.68×10^4 to 8.75×10^4	8.71×10^4	4.3

Table 4 The accuracies of the method.

Analyte	Background (mg/kg)	Spiked (mg/kg)	Found (mg/kg)	Recovery (%)	RSD (%)	Mean recovery (%)
RSP	ND [a]	1.00	0.92	92.0	4.9	90.5
		8.00	7.21	90.1	6.3	
		20.0	17.9	89.5	4.8	
CTD	ND	1.00	0.86	86.0	6.1	88.4
		8.00	7.31	91.4	8.7	
		20.0	17.6	88.0	9.2	
IDP	ND	1.00	0.71	71.0	6.1	73.7

		8.00	5.94	74.3	7.6	
		20.0	15.2	76.0	9.8	
HCT	ND	1.00	0.90	90.0	5.5	88.9
		8.00	7.71	96.4	6.2	
		20.0	16.1	80.5	9.8	
NDP	ND	1.00	0.94	94.0	6.1	95.1
		8.00	7.77	97.1	5.5	
		20.0	18.8	94.0	4.6	
VST	ND	1.00	0.95	95.0	3.1	99.5
		8.00	8.37	104.6	3.5	
		20.0	19.8	99.0	3.9	

[a] ND: not detected.

3.5 Method applications

The proposed method was successfully applied to the determination of the target chemicals in four real antihypertensive functional food samples. RSP was detected in a wine sample, with the content of 55.1 ± 0.9 μg/mL and RSP in the sample was qualitatively confirmed by LC-MS/MS (Fig. 4). No other target chemicals were detected in theses functional food samples.

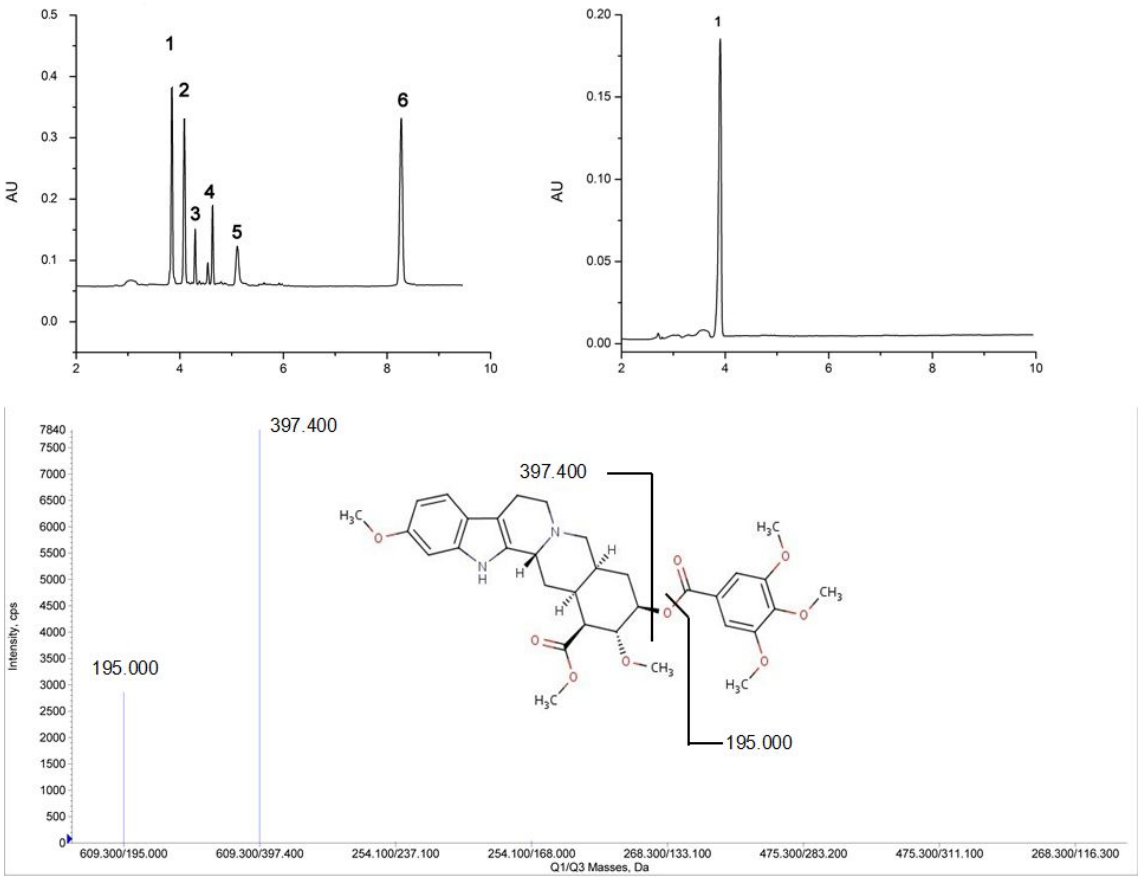


Fig. 4 RSP was detected in a wine sample (right) and the mass spectrum of RSP (below).

Peak indentifications: 1. RSP; 2. CTD; 3. IDP; 4. HCT; 5. NDP; 6. VST.

4 Conclusion

A new, simple, accurate, and precise MEKC capillary electrophoresis method was developed for the simultaneous determination of RSP, CTD, IDP, HCT, NDP and VST in antihypertensive functional foods. The developed method has distinct advantages regarding analysis time, injection volume and cost. The method provides an alternative method for monitoring the quality of antihypertensive functional foods.

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