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Determination of noradrenaline and dopamine in Chinese herbal extracts from *Portulaca oleracea* L. by high-performance liquid chromatography

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Abstract

A simple, rapid and accurate high-performance liquid chromatographic (HPLC) technique coupled with photodiode array (PDA) detection was developed for the simultaneous determination of noradrenaline (NA) and dopamine (DA) in Chinese herbal plant extracts from the different parts of *Portulaca oleracea* L. The effects of various parameters, such as sodium dodecyl sulfate (SDS) concentration, pH value and proportion of methanol on chromatographic behavior of the analytes (NA and DA) were investigated. Separation of NA and DA was achieved within 10 min by a mobile phase consisting of 70% (v/v) methanol and 0.02 *M* potassium dihydrogen phosphate solution, which contained 30 m*M* SDS and was adjusted to pH 3.0 with H_3PO_4. NA and DA showed good linear relationships in the range of 0.004–6.00 μg and 0.011–8.25 μg respectively. The correlation coefficients of the calibration curve for the analytes exceeded 0.999. The detection limits for NA and DA were 0.40 ng and 0.55 ng, at a signal-to-noise ratio of 3, respectively. Moreover, the optimized HPLC method was employed to analyze three different parts of *Portulaca oleracea* L.

Keywords: *Portulaca oleracea*; Pharmaceutical analysis; Plant materials; Noradrenaline; Dopamine; Catecholamines

1. Introduction

Portulaca oleracea L. (Chinese name Ma-Chi-Xian) is widely used in China not only as an edible plant, but also as a traditional Chinese herbal medicine for alleviating pain and swelling. It has the abilities of anti-bacteria, -virus, -antherasis, -caducity, -diabetes, and enhancing immunity [1]. Recent studies indicated that the consumption of *Portulaca oleracea* may help to reduce the occurrence of cancer and heart diseases [2], which may account for the fact that it is known as ''vegetable for long life'' in Chinese folklore. *Portulaca oleracea* contains abundant catecholamines, noradrenaline (NA) and dopamine (DA), and they were demonstrated to be the major bioactive constituents. The catecholamines (NA and DA) are generally considered to be the effective component for the treatment of shock. Some studies also show NA is a modulator of the immune system and postulated to have anti-cancer properties [3,4]. Thus, the increasing interests in catecholamines from *Portulaca oleracea* and their extensive effects have led to needs for analytical

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methods for their determination. In the literature, several methods have been established using high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) for NA and DA, but only for biological samples, such as blood, urine and brain tissue [5–9]. All these methods were based on the pre-column derivatization coupled with fluorescence detection or electrochemical detection. Because of the complex matrix and great different contents of desired components in crude herbs, simultaneously determining desirable components is difficult. Although analysis using CE was reported for the determination of NA and DA in the whole plant of *Portulaca oleracea* [10], a derivatization procedure was still needed.

In this paper, a simple, rapid and accurate HPLC method coupled with PDA detector was described for the analysis of NA and DA in extract from three different parts of *Portulaca oleracea*. The optimum conditions for the analytical method were investigated for the best resolution and highest sensitivity of detection. Then the proposed method was applied for determining NA and DA in the above plant.

2. Experimental

2.1. Reagents and chemicals

Standards of the analytes, noradrenaline (NA) and dopamine (DA), were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *Portulaca oleracea* was collected from Lanzhou, China. Methanol was of chromatographic grade and purchased from Tianjing Chemical Reagent Corporation (Tianjing, China). Potassium dihydrogen phosphate (KH_2PO_4), phosphoric acid (H_3PO_4), hydrochloric acid (HCl) and sodium dodecyl sulfate (SDS) were of analytical grade and purchased from Qing Huangdao, Tianjing and Beijing Chemical Reagent Corporation respectively (Qing Huangdao, Tianjing and Beijing, China, respectively). Distilled and deionized water was used for the preparation of all samples and solutions.

2.2. Chromatographic system

The HPLC system used was a Waters instrument (Milford, MA, USA), with a quaternary pump (Model Delta 600E), a photodiode array detector (Model 2996), manual injector and Waters Millennium[32] software for peak identification and integration. The chromatographic separation of analytes was performed on a Kromasil C_{18} column (5 μm, 250×46 mm I.D.) (Dalian Institute of Chemical Physics, Chinese Academy of Sciences; Dalian, China). Helium (He) was used for degassing the mobile phase. The temperature of the column during analysis was maintained at 40 °C. The injection volume was 10 μl each time.

2.3. Standard solution preparation

Standard stock solutions of the two analytes (NA and DA) with concentrations of 2 mg/ml were prepared in 0.1 *M* HCl. Then standard solutions with various concentrations were prepared by appropriate dilution of the stock solutions when needed.

2.4. Sample solution preparation

After being air-dried and crushed into powder, 0.5 g of the accurately weighed herbal sample was extracted with 25 ml of 0.1 *M* HCl solution in an ultrasonator for 1.5 h. The extract was then filtered through a filter paper and a 0.45 μm filter membrane to be ready for analysis. The recovery study followed the same procedure described above. As NA and DA are photosensitive, the sample preparation was carried out rapidly under a dim light working environment and kept in brown volumetric flasks.

3. Results and discussion

The analytes, NA and DA, have similar molecular structures except for a slight difference with or without a hydroxyl group on the side chain of the benzene ring, and their UV spectra showed strong absorbance bands at 280 nm, 220 nm and 203 nm. To avoid the influence of methanol at 220 nm and 203 nm, 280 nm was selected as detection wavelength. In the study, effects of SDS concentration, pH value and proportion of methanol on the separation were tested for achieving an optimum condition.

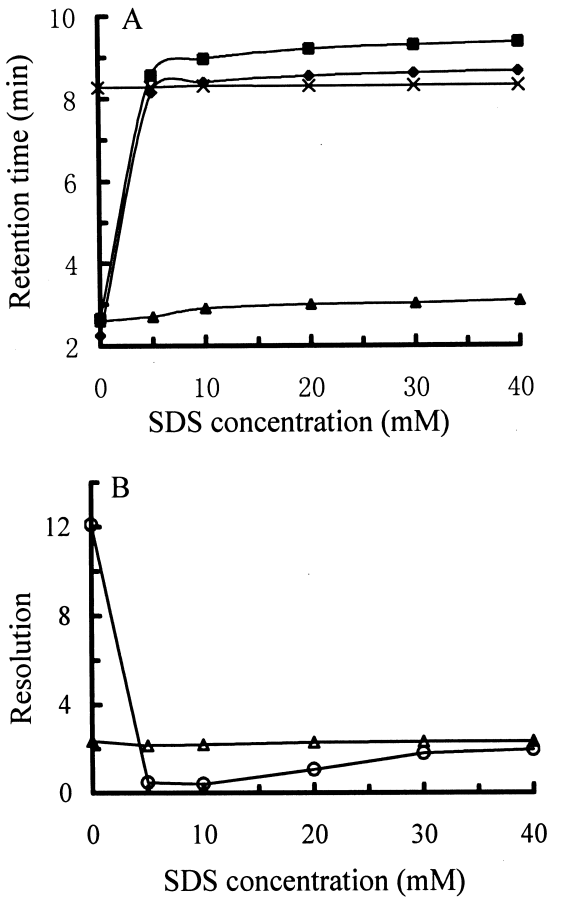

Fig. 1. Effect of SDS concentration on the retention time (A) and on the resolution of the adjacent peaks (B), DA (■), NA (♦), UN2 (×), UN1 (▲), NA−DA (△) and NA−UN2 (○). Conditions: 0.02 *M* KH_2PO_4 solution, 70% (v/v) methanol, pH 3.0.

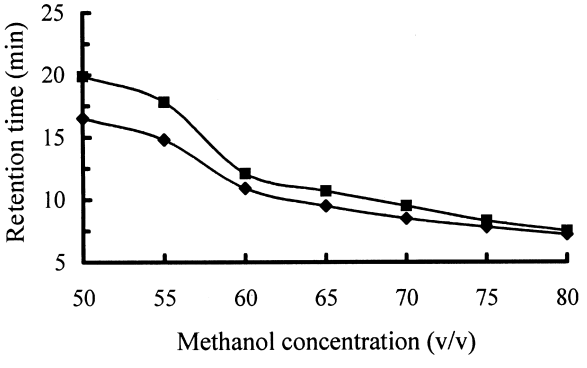

Fig. 2. Effects of proportion of methanol on the retention times of NA (♦) and DA (■). Conditions: 0.02 *M* KH_2PO_4 solution, 30 m*M* SDS, pH 3.0.

3.1. Effects of SDS on the separation

Various concentrations of SDS, ranging from 0 m*M* to 40 m*M*, were added to the pH 3.0 mobile phase consisting of 0.02 *M* KH_2PO_4 solution and 70% (v/v) methanol. Adding SDS gave rise to a change in the chromatographic behavior of NA and DA in the extract from the plant. Firstly, with increasing SDS concentration, the retention times of NA and DA increased, but the other unknown compounds kept at about 2 min and 8 min (the unknown mixture at about 2 min was marked as UN1 and at about 8 min as UN2) (see Fig. 1A). This phenomenon can be accounted for by the characteristic molecular structures of the NA and DA. Their strong hydrophilicity resulted in almost no retention on the reversed-phase column, but in the acid medium, they protonized and interacted with SDS added as an ion-pair reagent. All of these increased their retention times.

The results indicated that the concentrations of SDS had effects on the resolution not between NA and DA, but between the analytes and other unknown compounds. NA and DA could be greatly resolved from UN1 while adding 5–10 m*M* SDS to the mobile phase, but the NA peak was overlapped with the UN2 peak (Fig. 1B). With continuing increase of SDS concentration, the resolution between NA and UN2 increased. After addition of 30–40 m*M* SDS, they were separated completely. Therefore, 30 m*M* SDS was selected to enhance resolution and minimize separation time.

3.2. Effects of proportion of methanol on the separation

Before adding SDS to the mobile phase, the methanol content had no influence on the separation and retention time of NA and DA. Even without methanol, the mixture of NA and DA could be resolved very well, but in the extract of the plant they could not be separated from UN1 and eluted together with UN1 as a big overlap peak. After adding SDS, various proportions of methanol, ranging from 50% (v/v) to 80% (v/v) were tested for separating the analytes. The retention times of NA and DA were shortened with increasing methanol proportion, while the resolution decreased (see Fig. 2). When proportion of methanol exceeded 70%

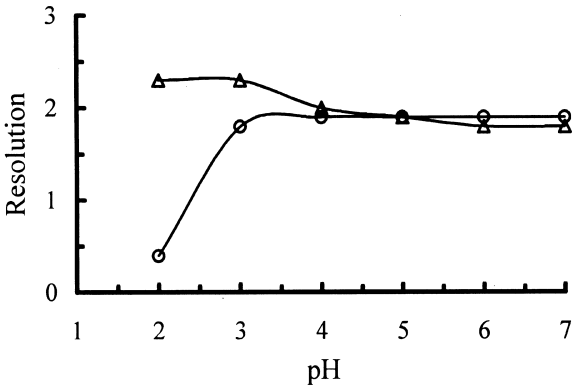

Fig. 3. Effect of pH value on the resolution of the adjacent peaks, NA–DA (△), NA–UN2 (○). Conditions: 0.02 *M* KH_2PO_4 solution, 30 m*M* SDS, 70% (v/v) methanol.

(v/v), NA and DA could not be completely separated. So 70% (v/v) methanol was used to keep separation efficiency and shorten analytical time.

3.3. Effects of pH value on the separation

Several mobile phase systems at different pH ranging from 2.0 to 7.0, in which the SDS concentration was 30 m*M* and the methanol proportion was 70% (v/v) were tested for separating the herbal extracts (Fig. 3). Fig. 3 revealed that the resolution of NA and DA was seldom affected by pH value, but at low pH value (pH<3.0), the resolution between NA and UN2 increased with increasing pH value. The results can be explained as follows: NA and DA were not influenced by pH value because in the extract procedure, 0.1 *M* HCl solution as extraction solution, NA and DA had already protonized completely, but UN2 was influenced by the degree of dissociation, which was dependent on the pH value of the mobile phase. Though its structure is unknown to us, we can suppose that it must be undissociated at low pH, and with increasing pH value, its dissociation and negatively charge increased, at pH 3.0 it dissociates completely with the shortest retention time and the best resolution from NA. Since the best separation efficiency and resolution for the analytes were achieved at pH 3.0, the mobile phase was adjusted to pH 3.0 for subsequent analysis.

Table 1 lists the regression equations, correlation coefficients of calibration curves and detection limits for the two analytes in optimum conditions. The peak area of the chromatogram was employed for quantitation of the analytes. The concentration ranges for calibration graphs were 0.004–6.00 μg for NA and 0.011–8.25 μg for DA. The correlation coefficients of the calibration graphs were 0.99997 for NA and 0.9996 for DA. In addition, the detection limits for the analytes ranged from 0.4 ng to 0.55 ng at a signal-to-noise ratio of 3. Our experimental results thus confirm that the HPLC method definitely possesses the advantages of high precision, high resolution and short analysis time for analyzing herbal extracts from different parts of *Portulaca oleracea*.

3.4. Extraction and determination of NA and DA in Portulaca oleracea L.

The interested components in herbal plant samples were identified by comparing both the retention times and the UV spectra of standards to those in actual samples. The analytes were further confirmed by spiking standards in actual samples. Fig. 4 illustrates the chromatograms of NA and DA, along with the extract of the leaves of *Portulaca oleracea*.

The influence of extraction solutions on the extraction of herbal plant samples was investigated. Water, methanol–water (1:1, v/v) and 0.1 *M* HCl solution were employed as extraction solution in this study. The 0.1 *M* HCl was better for the analytes exhibiting a significantly larger signal than those extracted by the others. This difference implies that the former has a better solubility and extraction power than the two latter for NA and DA. The

Table 1
Regression equations, correlation coefficients of linear calibration graphs and detection limits for the two analytes

Analyte	Regression equation	Correlation coefficient	Detection limit (ng)
Noradrenaline	$y=1.61\cdot10^6x+1701.20$	0.99997	0.40
Dopamine	$y=1.14\cdot10^6x-7560.95$	0.9998	0.55

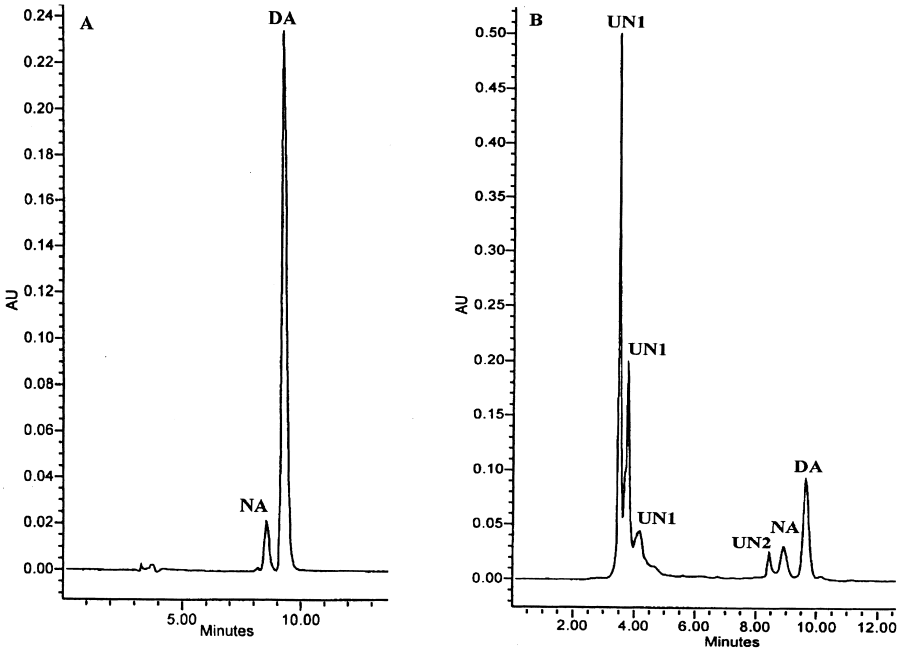

Fig. 4. Chromatogram of standard mixture (A) and the extract of the leaves of *Portulaca oleracea* (B). Condition: Mobile phase: 0.02 *M* KH_2PO_4 solution, 30 m*M* SDS, 70% (v/v) methanol, pH 3.0, 280 nm for detection wavelength.

analytes (NA and DA) were successfully extracted using 0.1 *M* HCl with an ultrasonic bath. Table 2 presents the recoveries of standards spiked in samples. The recoveries of the analytes were 92.3% and 96.9% with RSDs ($n=3$) of 2.09% and 1.56% respectively. The method was reproducible with inter-day RSDs ($n=5$) of 5.2% and 3.9% for NA and DA in the herbal medicine leaves respectively. The result demonstrated that the extraction method was adequate and appropriate for the analysis.

The comparisons of NA and DA in three different parts of the herbal medicine, respectively, leaves, stems and seeds are shown in Table 3. As shown in Table 3, the contents of the two compounds NA and DA in these three different parts were quite different. The contents of NA and DA in leaves were higher than those in stems and seeds. The amount of the analytes in the stems was lowest.

Table 2
Recoveries of the analytes spiked in the sample (leaf)

Analyte	Recovery[a] (%)	RSD (%)
Noradrenaline	92.3	2.09
Dopamine	96.4	1.56

[a] Values are means of triplicate determinations.

4. Conclusion

Although some methods have been reported for analyzing DA and NA from biological samples [11–14], to the best of our knowledge, there are no HPLC methods to analyze them from the extracts of herbal plant. The present study successfully develops a novel HPLC method for the simultaneous determination of NA and DA in the extract of three parts of *Portulaca oleracea* using 0.02 *M* KH_2PO_4 solution

Table 3
Contents of specific components in three different parts of *Portulaca oleracea* L.

Parts	Content (%)	
	Noradrenaline	Dopamine
Leaves	0.074	0.69
Stems	0.029	0.18
Seeds	0.054	0.59

adjusted to pH 3.0 containing 30 m*M* SDS and 70% (v/v) methanol. Determination of the bioactive constituents, DA and NA in *Portulaca oleracea* by the HPLC coupled with on-line PDA detection were relatively simple, rapid and accurate. The proposed method promises to be applicable to the quality control of traditional Chinese medicine.

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