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Journal of Chromatography B, 836 (2006) 108–110
JOURNAL OF CHROMATOGRAPHY B
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Technical note

Application of L-Amino acid in determination of Huperzine A by high performance liquid chromatography

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Received 4 October 2005; accepted 9 March 2006
Available online 29 March 2006

Abstract

A selective HPLC method for determination of Huperzine A in Huperzia serrata Extract has been developed and validated. Huperzine A was dissolved in 0.01 mol/L HCl, chromatographed on an Agilent Zorbax SB-C18 (150 mm × 4.6 mm i.d., 5 μm) column, with a mobile phase consisting of methanol-1 mM L-Lysine water solution (50:50, v/v), and detected at 310 nm. The UV peak areas were used for quantitation of Huperzine A content. The correlation coefficient (R^2) of the calibration was 0.9999 over the range of 1–25 μg/ml and intra- and interday precision over this range were not more than 2%. The method was successfully applied to characterize and determine the Huperzine A in Huperzia serrata Extract.

Keywords: Huperzine A; HPLC

1. Introduction

Huperzine A [(5R,9R,11E)-5-amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methanocycloocta-[B]-pyridin-2(1H)-one] (Fig. 1) is a Lycopodium alkaloid isolated from the Chinese herb Huperzia serrata (Thunb.) Trev. (Chinese name: Qian Ceng Ta) [1]. It is a potent, reversible acetylcholinesterase inhibitor, which crosses the blood-brain barrier smoothly, and shows high specificity for acetylcholinesterase. It has been approved as a drug for treatment of Alzheimer disease (AD) in China.

Recently, this extract of *Huperzia serrata* has captured the attention of researchers and medical professionals all over the word because of its application prospect. The quantiative analysis of Huperzine A in single-ingredient products using HPLC methods [2–4], in formulated products using HPLC/MS [5] and in dog serum using ion-pair RP-HPLC [6] or LC–MS–MS [7] have been reported previously, but these methods are generally not suitable for Huperzia serrata Extract, due to interference from other alkaloids. Although there are some methods for determination of Huperzine A content in Huperziaceae [8–10], further studies on the determination of Huperzine A in Huperzia serrata Extract are still necessary in order to increase the selectivity of Huperzine A from other alkaloids. The present paper describes a novel method to separate and quantitate Huperzine A in Huperzia serrata Extract, using reverse phase high performance liquid chromatography with a mobile phase consisting of methanol-L-Lysine water solution. The method was successfully applied to characterize and determine the Huperzine A in Huperzia serrata Extract.

2. Experimental

2.1. Chemicals and reagents

Huperzine A was purchased from National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Huperzia serrata Extract was provided by Hefei Tuofeng Company of Bioengineering Ltd. L-Lysine and L-Arginine were from Sigma Chemical Co. (St. Louis, MO). Methanol was HPLC grade, and all other chemicals were of analytical grade and used without further purification. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Equipment and chromatography condition

The HPLC system (Agilent 1100 series) consisted of a binary pump, a degasser, a UV detector and a manual injection with a

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doi:10.1016/j.jchromb.2006.03.018

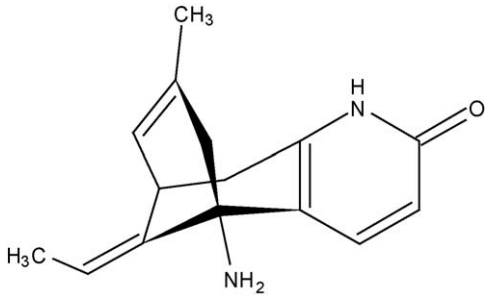

Fig. 1. The structure of Huperzine A.

20 μl fixed loop. The analytical column was an Agilent Zorbax SB-C18 (150 mm × 4.6 mm i.d., 5 μm). The mobile phase consisted of a mixture of methanol-1 mM L-Lysine (50:50, v/v) and delivered at a flow rate of 0.6 ml/min. The wavelength of UV detector was set at 310 nm.

2.3. Preparation of stock and standard solutions

Huperzine A was accurately weighted, transferred to volumetric flasks and dissolved in 0.01 mol/L HCl to make a stock solution of 100 μg/ml. The solution was stored at 4 °C and stable for at least 3 months. The stock solution was diluted with 0.01 mol/L HCl to certain concentration before use according to the analytical requirement.

2.4. Sample preparation

500 mg Huperzia serrata Extract were weighted into a 100 ml volumetric flask, 0.01 mol/L HCl were added, the mixture was sonicated for 10 min, and then cooled to room temperature. An aliquot was filtered through a 0.45 μm PTFE syringe filter into a HPLC vial for HPLC analysis. Each sample was extracted and analyzed in duplicate.

3. Results and discussion

3.1. Application of L-Amino acid

The mobile phases of different system and proportions were compared. Fig. 2 shows the chromatograms of study sample with three different mobile phases. The results indicated that the resolution between Huperzine A and impurities could be increased obviously adding L-Amino acids into the mobile phases. To choose the proper L-Amino acids, the solubility, UV absorbance and acidity/basicity were taken into consideration. The alkali L-Arginine and L-Lysine could be dissolved very easily in water. When they were added in the mobile phase, the selectivity was improved. First they adjusted the pH of the mobile phase just as triethylamine, the pH of the mobile phase consisting of methanol-1 mM L-Lysine water solution (50:50, v/v) was only 0.2 pH unit lower than that consisting of 0.02% triethylamine methanol solution-water (50:50, v/v), then the suitable retention time of Huperzine A was obtained. Secondly they may

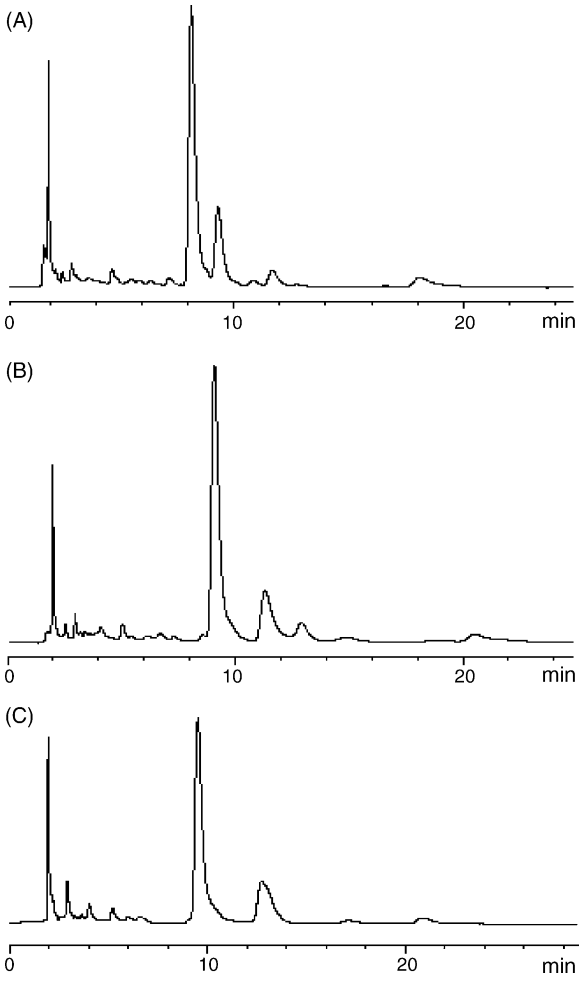

Fig. 2. Chromatograms of study sample with mobile phase consisting of: (A) 0.02% triethylamine methanol solution-water (50:50, v/v); (B) methanol-1 mM L-Arginine water solution (50:50, v/v); and (C) methanol-1 mM L-Lysine water solution (50:50, v/v).

interact with the alkaloids in the study samples, which alter the alkaloidas' retention characteristics. There maybe exist hydrogen bonding between L-Amino acids and the alkaloids because of amino group and carbonyl group, and dipole-dipole interactions because L-Amino acids are special dipolar ions. The mobile phase consisting of methanol-1 mM L-Lysine water solution (50:50, v/v) was chosen at last in terms of suitable retention time and complete resolution among Hupzine A and impurities in the sample.

3.2. Method validation

3.2.1. Specificity

Representative chromatograms of standard sample and a study sample containing a low concentration of Huperzine A are shown in Fig. 3. The results indicated that the method was specific for determining Huperzine A under the chromatographic conditions employed. The peak location of Huperzine was not interfered by the other compounds in Huperzia serrata Extract.

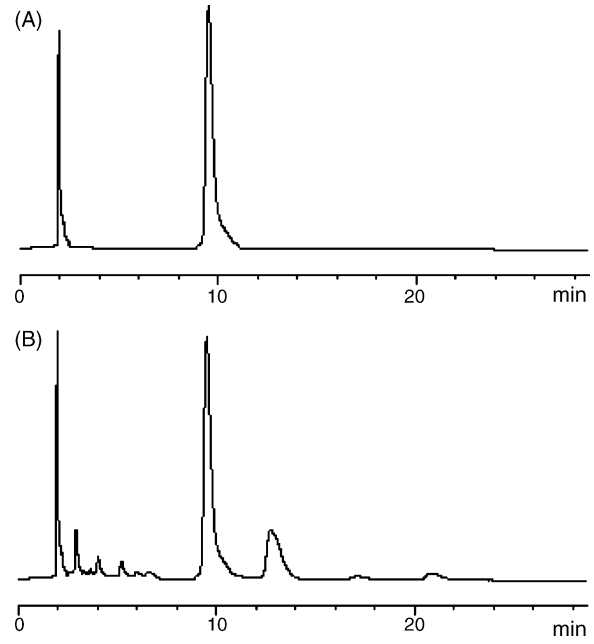

Fig. 3. Chromatograms of: (A) standard sample and (B) study sample.

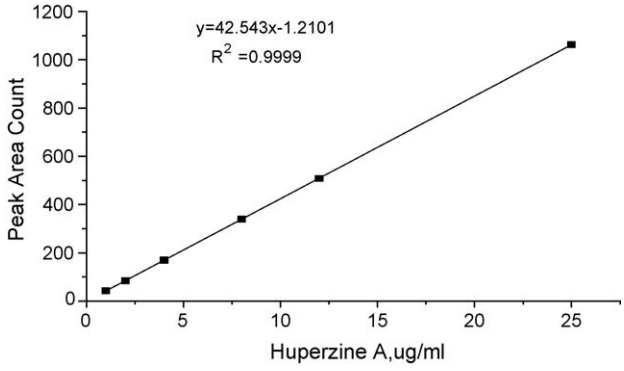

Fig. 4. Typical linear calibration for Huperzine A.

3.2.2. Calibration curves

The calibration curve for Huperzine A was constructed by analyzing a series of Huperzine A standard samples in the concentration range from 1 to 25 μg/ml. The calibration curve of Huperzine A was linear over the concentration range from 1 to 25 μg/ml. The regression equation was $y = 42.543x - 1.2101$. Linear regression analysis of the data yielded a correlation coefficient (R^2) of 0.9999 (Fig. 4).

3.2.3. Precision

The intra- and inter-day precision were obtained by analyzing the standard samples at concentrations ranging from 1 to 25 μg/ml in five replicates within 1 day and on 5 consecutive days, respectively. The intra-day R.S.D. ranged from 1.08% to 1.71%. For the same concentration range, the inter-day R.S.D. ranged from 1.23% to 1.86%, respectively. The results were shown in Table 1.

Table 1
Precision for the determination of Huperzine A

Added concentration (μg/ml)	Found concentration (μg/ml)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
1	1.01	1.71	1.86
12	12.1	1.12	1.23
25	25.5	1.08	1.75

3.2.4. Recovery

The recovery test was carried out using standard addition method. Suitable amounts of Huperzine A were added to 500 mg Huperzia serrata Extract of known Huperzine A content, the mixture was processed as described under sample preparation and analyzed using the proposed procedure. The recovery of Huperzine A was 99.8% ($n = 5$).

4. Conclusion

A HPLC method for determining Huperzine A in Huperzia serrata Extract has been developed and validated. The HPLC method described is simple, accurate and selective. The selectivity was sufficient to determining Huperzine A content without any interference from the impurities.

Acknowledgement

This work was supported by Grant 20136020 from the National Natural Science Foundation of China.

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