Available online at www.sciencedirect.com

ScienceDirect

IERI Procedia 5 (2013) 351 – 356

Procedia IERI

www.elsevier.com/locate/procedia

2013 International Conference on Agricultural and Natural Resources Engineering

Pre-column Derivatization RP-HPLC Determination of Amino Acids in Asparagi Radix before and after Heating Process

Zhihong Shi, Hui Li, Zhimin Li, Junda Hu and Hongyi Zhang*

Key :Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding 071002, China

Abstract

A pre-column derivatization RP-HPLC assay was established for the simultaneous determination of 12 kinds of amino acids in Asparagi Radix before and after heating process. PITC was used as pre-column derivatization reagent. The obtained amino acid derivatives were separated on C_{18} column (250 × 4.6 mm i.d., 5μm) by using gradient elution with 0.1 mol/L pH6.5 acetate buffer solution-acetonitrile (93:7,v/v) as mobile phase A and acetonitrile-water (4:1,v/v) as mobile phase B. 12 kinds of amino acids could be detected at 254nm in 40min with a flow rate of 0.9mL/min. Linear range was between 0.5-500μg/mL and average recovery was between 75.4% -100.1 %. Experimental results showed that the amino acid content in Asparagi Radix was decreased after heating process.

*Keywords:*Amino acid, Asparagi Radix, Pre-column derivatization, PITC, HPLC

1. Introduction

Asparagi Radix (Chinese name Tiandong) is the root of *Asparagus cochinchinensis* (Lour.) Merr. family *Liliaceae*, and its pharmaceutical effects include moisturizing the lung, clearing away heat and benefiting the kidney[1]. Asparagi Radix is rich in glucose, fructose, a variety of oligosaccharides, asparagine and nearly 20 kinds of amino acids. For the processing of Asparagi Radix, one of the methods is to torrify it to yellow [2].

* Corresponding author. Tel.: +86-312-5079357; fax: +86-312-5079525.
E-mail address: hyzhang@hbu.edu.cn.

doi:10.1016/j.ieri.2013.11.115

Yet as far as we know, no work has been published on the comparison of the amino acid content in Asparagi Radix before and after heating process.

For the determination of amino acids, several methods have been employed, such as amino acid analyzers [3], capillary electrophoresis [4] and high-performance liquid chromatography[5]. The combination of precolumn derivatization and HPLC separation followed by fluorescence detection or UV detection is the most convenient and commonly used approach. Nearly 10 kinds of reagents have been employed for the derivatization of amino acids, such as o-phthalaldehyde(OPA)[6], 2,4- dinitrofluorobenzene (DNFB)[7], phenyl isothiocyanate (PITC)[8] and fluorenylmethyloxycarbonyl (FMOC)[9] and so on. In this paper, PITC was used as derivative reagent for the HPLC determination of 12 kinds of amino acids in Asparagi Radix. The contents of amino acids were compared before and after Asparagi Radix were processed at different temperatures.

2. Experimental

2.1 Instrument and reagents

An LC-20AD Chromatograph was used for analysis, which was equipped with a UV/VIS detector and a CBM-10Avp plus LC Solution Lite software (Shimadzu, Japan).

12 kinds of amino acid reference substances (aspartic acid, serine, glycine, glutamic acid, alanine, valine, methionine, isoleucine, threonine, phenylalanine, lysine and tryptophan) were biochemical reagents (Sigma); PITC (Sinopharm Chemical Reagent Co., Ltd., China), triethylamine (Tianjin Fu Chen Reagent Factory, China), Acetonitrile was HPLC reagent (Tianjin Kermel Chemical Reagent Co., Ltd., China). Unless otherwise indicated, the reagents used were of analytical purity. Water was doubly distilled. Asparagi Radix was obtained from local drug store (Baoding, China).

2.2 Preparation of Solution

2.2.1 Preparation of mobile phase

Mobile phase A: 15g sodium acetate was dissolved in 1700mL water with acetic acid adjusted to pH 6.5, water was added to 1850mL and finally 140 mL acetonitrile was added. The thoroughly mixed solution was filtered through 0.45μm membrane.

Mobile phase B: acetonitrile-water (4:1, v/ v), filtered through 0.45μm membrane.

2.2.2 Preparation of derivatization reagents

1.0 mol·L^{-1} triethylamine in acetonitrile: 417μL of triethylamine was mixed with 2583μL of acetonitrile.

0.1 mol·L^{-1} PITC in acetonitrile: 36μL of PITC was mixed with 2164μL of acetonitrile.

2.2.3 Preparation of amino acid standard solutions

0.1g of each amino acid was accurately weighed and placed in 100mL volumetric flask, water was added to dissolve the amino acids and the solution was diluted to the mark. The obtained 1mg/mL standard stock solution was kept at 4 °C.

2.2.4 Treatment of Asparagi Radix