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Short communication

Ultrasonic-assisted precolumn derivatization-HPLC determination of acrylamide formed in Radix Asparagi during heating process

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ABSTRACT

An ultrasonic-assisted precolumn derivatization-HPLC method was established and validated for the determination of acrylamide formed in traditional Chinese herb Radix Asparagi during heating process. This method entails extraction with water, ultrasonic-assisted derivatization with 2-mercaptobenzoic acid. The final extracted acrylamide derivative was separated on C_{18} column by using a mixture of acetonitrile and acetic acid (1 g L^{-1} water solution) (20:80, v/v) as mobile phase. The flow rate was 0.7 mL min^{-1} and the detection wavelength was set at 238 nm. Factors influencing the derivative reaction were evaluated and the optimum derivatization conditions were as follows: molar ratio of derivative reagent to acrylamide was 35:1, pH 8–12, ultrasonic-assisted reaction time was 100 min. The calibration curve of acrylamide showed good linearity in the range of 0.015–4.5 μg mL^{-1} with correlation coefficient of 0.9999. The limit of detection was estimated to be 25 μg kg^{-1} based on the signal-to-noise ratio of 3 recorded at 238 nm. Recovery of acrylamide from the sample was 106.6 ± 6.6%. Relative standard deviation of five duplicate determinations for the same sample solution was 1.59%.

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1. Introduction

The potential health risk of acrylamide in food has drawn worldwide attention since April 2002 [1]. Acrylamide formation was found to occur during the browning process by Maillard reaction of reducing sugars with asparagine at temperatures above 120 °C [2,3]. The presence of acrylamide in carbohydrate and asparagine-rich food made us think about the processing of Chinese herbs. As we know, many kinds of Chinese herbs are originated from plant roots, some of them need to be processed by heating at proper temperature to make it more fit to be used for the treatment of certain diseases [4]. Whether or not acrylamide will be produced during the heating process is a question that attracts our research interest. In this work, we selected Radix Asparagi (Chinese name Tiandong), which is rich in asparagine and sugar [5] as research model. Radix Asparagi is the root tuber of *Asparagus cochinchinensis* (Lour.) Merr., family *Liliaceae*, and it has the pharmaceutical effect of clearing away heat, moisturizing the lung and benefiting the kidney [6]. It is reported that one of the processing methods for Radix Asparagi is to torrify it to yellow [7]. The aim of this paper is to establish a reliable, selective, interference avoiding analytical method for the quantitation of acrylamide in heat-processed Radix Asparagi samples based on LC-UV, which can be easily adopted by common analytical laboratories. To resolve the problem of acrylamide's poor retention on conventional RP-HPLC column and to avoid the interference of co-extractives, derivatization of acrylamide was performed based on the reaction with 2-mercaptobenzoic acid [8]. To shorten the derivatization time, ultrasound was employed in our work to accelerate the reaction and various factors influencing the reaction was evaluated and optimized.

2. Experimental

2.1. Standard

Acrylamide (>99.9%) was purchased from Amresco (Solon, Ohio, USA). Stock solution of acrylamide (0.1 mg mL^{-1}) was prepared by dissolving the compound in doubly distilled water. Working standards were prepared by diluting the stock solution of acrylamide to concentrations of 0.015, 0.15, 0.30, 1.5 and 4.5 μg mL^{-1} with doubly distilled water. Stock solution was stored at 4 °C for a maximum of 4 weeks.

2.2. Reagents and materials

2-Mercaptobenzoic acid (≥98.0%) was obtained from Jinlong Chemical Reagents Co. Ltd. (Beijing, China). A solution of 2-mercaptobenzoic acid was prepared by dissolving 154 mg of 2-mercaptobenzoic acid in 10 mL of 1 mol L^{-1} NaOH solution.

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Traditional Chinese herb Radix Asparagi was purchased from Yixiaotang drug store (Baoding, China) and its pharmacognostic identification was done by Professor Ma Xiaoli from Health Science Center of Hebei University.

2.3. Apparatus

HPLC analysis was performed on an LC-10ATvp plus liquid chromatograph (Shimadzu, Japan) which consisted of an LC-10ATvp plus pump, a Rheodyne model 7725i injection valve (sample loop 20 μL) and an SPD-10Avp plus multi-wavelength detector. The chromatographic data were recorded and processed with a CBM-10Avp plus LC Solution Lite software. The analytical column was a Diamonsil C_{18} (150 mm × 4.6 mm i.d., 5 μm) column.

2.4. Heating process for Radix Asparagi

The raw material of traditional Chinese herb was cut into slices by stainless steel knife. Then the pieces were put in a culture dish in a thin layer and heated in a thermostatic-electric oven at 100 °C, 140 °C and 200 °C for 20 min, respectively. The heat-processed Radix Asparagi was pulverized into powder and homogenized.

2.5. Extraction and derivatization of acrylamide

0.5000 g of the pulverized sample was accurately weighed and put into a centrifuge tube. 10 mL of water was added and the sample was extracted for 10 min on a vortex oscillator. Then the homogenate was centrifuged at 5000 rpm for 10 min, 5 mL of the supernatant was transferred into a conical flask and the solution was adjusted to pH 8 ± 0.3 by adding aqueous sodium hydroxide (1 mol L^{-1}). After the addition of 0.5 mL 2-mercaptobenzoic acid solution for derivatization (pH was increased to about 10), the conical flask was sealed with a glass plug and the reaction mixture was treated with ultrasound in the dark for 100 min. The excess derivative reagent was then removed by adding 1 mL of saturated lead (II) acetate water solution, and after centrifugation at 5000 rpm for 10 min, the supernatant was transferred into another centrifuge tube and was acidified to pH 1.5 ± 0.3 with hydrochloric acid (5 mol L^{-1}). Then after centrifugation at 5000 rpm for 10 min, the supernatant was transferred into a centrifuge tube and was extracted with ethyl acetate (2 × 4 mL). After extraction, the organic phase was evaporated at 70 °C. The residue was redissolved in 250 μL of methanol, a 10 μL portion of the sample was injected for LC-DAD or LC-UV analysis.

2.6. HPLC analysis

Separation was carried out on a Diamonsil C_{18} (150 mm × 4.6 mm i.d., 5 μm) column. A mixture of acetonitrile and acetic acid (1.0 g L^{-1} water solution) (20:80, v/v) was employed as mobile phase. The flow rate was 0.7 mL min^{-1}; the detection wavelength was set at 238 nm. Peaks in the chromatograms were identified by comparing the retention time and UV spectrum with those of the authentic acrylamide derivative. Peak area was used for quantification.

3. Results and discussion

3.1. Optimization of the derivative conditions

3.1.1. Influence of the ultrasonic-assisted reaction time

The effect of ultrasonic-assisted reaction time on the derivatization of 100 μg acrylamide standard was studied by varying the reaction time from 40 to 130 min. Experimental result showed that 100 min was enough to complete the reaction. To achieve the same reaction efficiency, the common agitation method described in literature [8] needs more than 3 h. The ultrasonic cavitation phenomenon might be the key factor to accelerate the reaction, in which microbubbles formed and broke and energy was released in the liquid medium. The released energy could help to open the chemical bonds and accelerate the derivative reaction.

3.1.2. Influence of molar ratio of the derivative reagent to acrylamide

The effect of the amount of derivative reagent on the derivatization of 100 μg acrylamide standard was investigated by varying the volume of 2-mercaptobenzoic acid solution from 0.02 to 0.5 mL. Experimental result indicated that to fulfill the derivatization completely, molar ratio of 2-mercaptobenzoic acid to acrylamide should be controlled at least above 17.5:1. In this paper, we selected molar ratio of 35:1 for further study.

3.1.3. Influence of pH

The influence of pH on the efficiency of the derivatization was evaluated by varying the pH of the solution from 7 to 14. It was found that the derivatization was affected enormously by the pH of the solution and the optimal pH range was pH 8–12. So pH 10 was selected as the optimum pH value for the reaction.

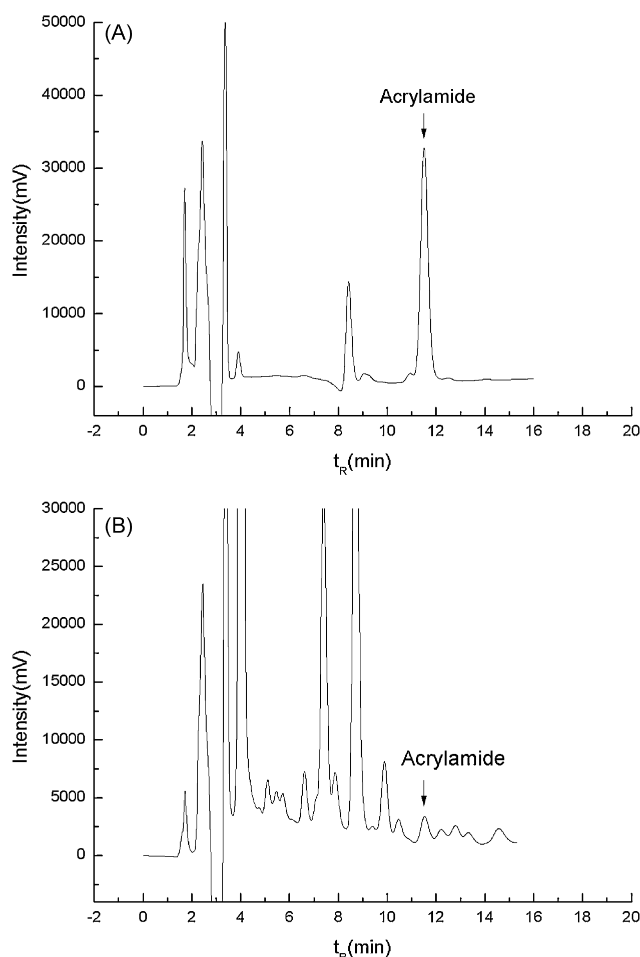

Fig. 1. Typical chromatograms for derivative product of authentic acrylamide standard (A) and derivative product of torrified Radix Asparagi processed at 140 °C (B).

Table 1
Analysis of acrylamide in raw Radix Asparagi and heat-processed Radix Asparagi.

Sample	Raw Radix Asparagi	Heat-processed Radix Asparagi		
		100 °C	140 °C	200 °C
Acrylamide content[a] (mg/kg)	N.D.	N.D.	2.32	4.14

[a] $n = 3$.

3.2. Linear range and limit of detection

Calibration curve was generated by derivatizing 5 mL of acrylamide standard solution (0.015, 0.15, 0.3, 1.5, and 4.5 $\mu g\, mL^{-1}$) according to the method described in Section 2.5. The final solution was analyzed and data were recorded. The calibration graph was plotted based on linear regression analysis of peak area of the derivative product of acrylamide (Y) versus concentration of acrylamide (X). The regression equation for acrylamide was $Y = 5929.89 + 349679.44X$ and the correlation coefficient was $R = 0.9999$. The limit of detection (LOD) was calculated to be 25 $\mu g\, kg^{-1}$ based on a signal-to-noise ratio of 3.

3.3. Repeatability and reproducibility test

To test the repeatability of the method, the same sample solution was injected for five duplicate LC analyses. The relative standard deviation (R.S.D.) for the determined content of acrylamide was 1.59% ($n = 5$).

The reproducibility test was carried out by treating three duplicate fried Radix Asparagi samples with the established method. The R.S.D. for the determined content of acrylamide was 4.32% ($n = 3$).

3.4. Recovery test

Recovery experiment was carried out by spiking acrylamide into heat-processed Radix Asparagi sample containing known amount of acrylamide and the spiked sample was treated according to the sample preparation procedures and determined by the established LC method. The recovery was found to be $106.6 \pm 6.6\%$ based on three duplicate experiments.

3.5. Determination of acrylamide formed in Radix Asparagi during heating process

The acrylamide content in heat-processed Radix Asparagi and raw Radix Asparagi are shown in Table 1. Typical chromatograms for derivative product of authentic acrylamide standard and derivative product of fried Radix Asparagi (140 °C) are shown in Fig. 1. It could be seen that the determination of acrylamide was not interfered by other co-existing substances.

4. Conclusions

The results of this study revealed that a conventional LC instrument coupled to precolumn derivatization can also be used accurately and precisely, as an alternative to tandem LC–MS methods for the determination of acrylamide. Based on the established method, content of acrylamide in heat-processed Chinese herb Radix Asparagi was reported for the first time. Whether or not the acrylamide formed in heat-processed Chinese herbs will do harm to human health or have special effect on its pharmacological function is a question deserving further study.

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