Abstract: An analytical method about the determination of natural Vitamin E and the benzopyrene (BaP) in the Natural Vitamin E raw materials has been developed by High Performance Liquid Chromatography/Fluorescence Detection (HPLC/FLD). After the purification and enrichment by Cleanert® Bap-3 SPE column series connection with Cleanert® Silica SPE column, the Natural Vitamin E and benzopyrene can be successfully separated by isocratic elution (acetonitrile:water=80:20) on Venusil® ASB C18 column. The spiked recovery of BaP in Natural Vitamin E samples were in the range of 81.3-87.0%, and the calibration curve has shown good linearity (r=0.9996). The LOQ of this method for benzopyrene was 0.5 µg/g, and the detected results of benzopyrene in the natural vitamin E was consistent with GC-MS.

Keywords: Natural Vitamin E; Benzopyrene; HPLC/FLD

INTRODUCTION

Benzopyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) which contains five benzene rings and has a strong carcinogenic effect. BaP is commonly found by environment pollution or as a by-product in the industrial process of petroleum and chemical. It was also found in grilled and broiled food.

In order to evaluate the content of benzopyrene in the Natural Vitamin E, we developed a rapid, simple and accurate method for the quantitative detection of Natural Vitamin E products and the benzopyrene which in the Natural Vitamin E products or raw materials. This method has a good impurities effect and the detection time was about 13min.

EXPERIMENTAL

1. Materials and Reagents

Hexane, dichloromethane and acetonitrile were of chromatographic reagent grade (Honeywell, Germany). Benzopyrene was purchased from Sigma (St. Louis, MO, USA). Cleaning Cleanert® Bap-3 SPE column series connection with Cleanert® Silica SPE column was activated by 5 mL dichloromethane and 5 mL hexane, and then the purified liquid was transferred to the SPE columns at a flow rate of 2 mL/min. Washing sample vials with 1 mL of hexane again, and then also transferred the wash liquor to the SPE columns. After washing the SPE columns with 10 mL of hexane, the Cleanert® Silica column was discarded. Finally, the Cleanert® Bap-3 column was washed with 5 mL of hexane and eluted with 5 mL dichloromethane. The eluate was collected and evaporated to dryness under a stream of nitrogen (400 °C). The dry residue was reconstituted in 1 mL of acetonitrile. The sample was filtrated via 0.45 µm filter membrane and for detection.

2. Instrumentation

Liquid chromatography with fluorescence detection (Shimadzu, Japan). Ultrasonic cleaner and nitrogen analyzer was from Bonna-Agela Technologies (Tianjin, China).

3. HPLC/FLD Conditions

Identification and quantification of the target compounds were carried out by using an Liquid Chromatography with fluorescence detector. Chromatographic separation of natural vitamin E and benzopyrene was achieved on Venusil® ASB C18 column. The mobile phase consisted of water and acetonitrile (20:80(V/V)), an isocratic elution program at 1 mL/min for 20min was used to achieve the separation, while the injection volume was 20 µL. The emission wavelength was set at 406 nm and the excitation wavelength was 384 nm.

3. Accuracy and Precision

In order to verify the accuracy and precision of this method, the benzopyrene standards solution was added to the natural vitamin E samples. The accuracy of the method was assessed by the recovery tests, as it was shown in Table 1, the average recovery was 81.3-87.0% for benzopyrene, and the RSD were in the range of 1.37-2.18%.

Table 1. The average recovery of benzopyrene (n=3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Averaage</th>
<th>RSD</th>
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<tbody>
<tr>
<td>61.87</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td>60.94</td>
<td>2.10</td>
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RESULTS AND DISCUSSION

1. Linearity and Limit of Quantitation

The Linearity and limit of quantitation (LOQ) of the proposed method were calculated after establishment of the optimum conditions for the benzopyrene standards detection. Calibration curves were constructed by plotting the peak area of benzopyrene versus the concentration of benzopyrene. The standards at the following concentrations: 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 ng/mL, were used to assess the linearity, and the linear equation was y=37658x-47093, correlation coefficient r=0.9996. The LOD of benzopyrene was 0.5 µg/g.

2. The Detection of the Benzopyrene in Natural Vitamin E Products or Raw Materials

The developed method was successfully used for the determination of benzopyrene in natural vitamin E products or raw materials. This method has a good impurities effect and the pre-treatment was very simple, rapid and accurate. From the Figure 1, we can see the benzopyrene and natural vitamin E were separated perfectly. The retention time of benzopyrene and natural vitamin E were about 11.22 min and 5.58 min respectively.

CONCLUSION

In this paper, we described a simple and accuracy HPLC/FLD method for the fast determination of benzopyrene in natural vitamin E raw materials. It has been applied in the determination of the benzopyrene in the grease samples like vegetable oil, and the results were in agreement with the results of GC-MS detection.