Determination of fungal toxin residues in bergamot pear by UPLC-MS/MS

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Abstract. The aim of this work was to establish a method that the residues of fungal toxin including citrinin, alternariol and alternariolmethylether in bergamot pears were determined by UPLC-MS/MS. Samples were extracted by acetonitrile, using NaCl and anhydrous Magnesium Sulfate to remove the water, and C18 powder was used to purify and get rid of miscellaneous, then used organic membrane to filter the samples. Results showed that the detection limits of citrinin, alternariol, and alternariolmethylether were 0.3 µg/kg, 0.5 µg/kg and 0.2 µg/kg respectively. The average recovery rates of alternariolmethylether, alternariol, citrinin were 79% ~ 101%, and the relative standard deviations were between 0.3% ~ 4.5%.

1. Introduction

The bergamot pear is easily contaminated by Penicillium, Streptomyces and Aspergillus during storage and transportation, and the pathogenicity of the fungus is the mycotoxin produced. Streptomyces is widely distributed in vegetables, cereals, fruits and other agricultural products. It is an important plant pathogen[1-3]. Because it can grow at low temperature, it can cause the corruption of refrigerated vegetables and fruits. Under suitable conditions, Streptomyces can produce a variety of metabolites, the main products of which are streptosporin and streptophenol methyl ether.

Citrinin is a secondary metabolite mainly produced by Penicillium, Aspergillus and other fungi, which is widely found in many kinds of fruits and their products, and has strong toxic effect on animal cell tissue, and has potential carcinogenicity and mutagenicity[4-6].

The main methods used in the detection of mycotoxins in fruits are thin layer chromatography, gas chromatography, liquid chromatography, enzyme-linked immunoassay, liquid chromatography tandem mass spectrometry, etc[7-12]. Liquid chromatography is one of the most widely used methods, but its detection Pre-treatment is more troublesome which are needed to carry out separation, purification and concentration steps. Some mycotoxins also need to be derived treatment, sometimes the sample interference will appear false positive. The detection time of enzyme linked immunoassay is short, but the sensitivity is relatively low, so it is easy to appear false positive. The sample matrix interference by gas chromatography is too large and the accuracy is not high. Liquid chromatography tandem mass
spectrometry has the advantages of strong anti-interference, qualitative and quantitative accuracy, high sensitivity and so on. At present, there is no standard and literature about simultaneous detection of these three mycotoxins in China. Therefore, this study intends to explore three methods suitable for the detection of three mycotoxins in fruits such as orange penicillin, streptosporin methyl ether and streptosporin, and provide technical and data support for the development of national standards.

2. Materials and Methods

2.1. Instruments and materials
WatersTQD ultra-high performance liquid chromatography tandem mass spectrometer, tissue homogenizer, centrifuge, vortex mixer. Ultra-pure water, methanol, acetonitrile are all chromatographic pure, citrinin, alternariol, alternariolmethylether and alternariolmethylether standard (purity >95%).

Preparation of standard working solution: accurately take appropriate amount of standard reserve solution, dilute with acetonitrile-water solution to mixed standard working solution. Acetonitrile-water solution: take 50 mL acetonitrile and 50 mL water.

2.2. Instrument condition
Chromatographic column: ACQUITY UPLC BEHC18 (50mm×2.1mm,1.7μm); column temperature :40℃; injection volume :10μL; flow rate :0.3 mL/min; the mobile phase system was A: acetonitrile, B: 5mmol/L ammonium acetate. gradient elution procedure:0~2.5 min, A from 20% to 90%;2.5~3 min, A from 90% to 20%;3~4 min, A20% maintain 1 min.

Liquid chromatography tandem quadrupole mass spectrometry: distribution spray ion source (ESI); ionization mode: positive ion mode scanning (ESI), negative ion mode scanning (ESI-); Mass spectrum scanning mode: multi-reaction monitoring (MRM); ionization voltage is 3 kV, ion source temperature is 110℃, desolvent temperature was 350℃, desolvent gas flow rate was 700 L/h, cone hole backblow gas flow rate was 50 L/h.. the monitoring ion, cone hole voltage and collision voltage mass spectrometry parameters were shown in table 1.

| compound          | ion mode | mother ion (mass charge ratio) | ion (mass charge ratio) | cone hole energy (V) | collision energy (V) |
|-------------------|----------|--------------------------------|-------------------------|----------------------|----------------------|
| alternariolmethylether | ESI-     | 271.5                          | 227.7                   | 38                   | 30                   |
|                    |          |                                | 255.7*                  | 38                   | 21                   |
| alternariol       | ESI-     | 257.5                          | 146.7                   | 47                   | 30                   |
|                    |          |                                | 212.7*                  | 47                   | 25                   |
| citrinin          | ESI+     | 251.3                          | 90.6                    | 40                   | 25                   |
|                    |          |                                | 232.7*                  | 40                   | 25                   |

Note :* Quantitative ions

3. Methods

3.1 Sample extraction and purification
The samples (5*0.01 g) were weighed into 50 mL centrifugal tube which were added 10 mL acetonitrile, 2.0 g anhydrous magnesium sulfate and 1.0 g NaCl. Then oscillation extraction 2min in the vortex mixer, and centrifugation for 7 minutes with 10000 r/min speed. Taked 2.0 mL supernatants which were added 30 mg C18 powder. Then vortex 2 min, and centrifugation for 7 min, using 0.22μm organic filter membrane filtration for analysis.
3.2 Results and discussion

3.2.1 Linear range and detection limit
Mix standard working solutions of citrinin, alternariol and alternariolmethylether at five levels (5, 10, 40, 60 and 100 ng/mL) were analysed by the above conditions, the ratios of peak area(Y) of measured component to the mass concentration (X) were calculated. The results showed that the linear relation between peak area and mass concentration of the citrinin, alternariol and alternariolmethylether were good in the context of 5~100 ng/mL. Table 2 represented the regression equations, correlation coefficients and instrument detection limits (S/N=3). The total ion flow diagram of the standard solution is shown in figure 1.

Table 2 Linear ranges, regression equation and detection limit

| compound          | linear range (ng/mL) | regression equation     | correlation coefficient | detection limit (μg/kg) |
|-------------------|----------------------|-------------------------|-------------------------|-------------------------|
| citrinin          | 5-100                | y=69.815x+256.36        | 0.9997                  | 0.3                     |
| alternariol       | 5-100                | y=32.456x+17.0269       | 0.9996                  | 0.5                     |
| alternariolmethylether | 5-100              | y=404.356x+2241.531    | 0.9993                  | 0.2                     |

Fig.1 The total ion flow diagram of citrinin, alternariol and alternariolmethylether

3.2.2 Precision and accuracy of the method
Choosing bergamot pear without citrinin, alternariol and alternariolmethylether drug residue as blank matrix, and set three different addition levels (2ug/kg, 10ug/kg, 20ug/kg), and each level of 3 parallel samples, the addition recovery rate and its relative standard deviation were calculated separately to investigate the accuracy of the method. The results were shown in Table 3. It can be seen from Table 3 that the recovery rates of these three mycotoxins were very high, which indicated that the analytical method had good accuracy and precision.

Table 3 Recovery

| compound    | blank μg/kg | addition quantity μg/kg | measurements μg/kg | addition recovery rate(%) |
|-------------|-------------|-------------------------|--------------------|---------------------------|
| citrinin    | 0.00        | 2                       | 1.85, 1.92, 1.87   | 94                        |
### 3.2.3 Actual sample testing

We randomly selected 50 bergamot pears from a farmer's market and taobao to determine the residues of citrinin, alternariol, and alternariolmethylether. The results showed that there were 4 bergamot pears which were determined citrinin, its contents were between 0.56 μg/kg~10.5μg/kg, there were 5 bergamot pears which were determined alternariolmethylether, content between 2.9 μg/kg~18.6 μg/kg; 4 samples detected positive sample content between 0.87 and 28.15 μg/kg. All the other samples were not detected in this experiment, some of the fragrant pear samples were corrupted, and some of the samples were squeezed out of the juice. the exudation of fruit nutrients may aggravate the reproduction of streptomyces, which makes the AME,AOH,CIT content higher.

### 4. Conclusions

In this paper, the ultra-high performance liquid chromatography tandem mass spectrometry (HPLC) method for the residue of orange penicillin, streptozotocin and streptosporin methyl ether was established. The method can obtain better sensitivity, precision and accuracy. It has the characteristics of simplicity, rapidity and strong applicability, and is suitable for the determination of orange penicillin, streptosporin and streptophenol methyl ether residues in fragrant pear. Experimental results show that rotten fragrant pear will make the AME,AOH,CIT content on the high side, there is a certain risk.

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