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RESEARCH PAPER

Simultaneous Determination of Plant Growth Regulators in Fruit by Ultra-performance Liquid Chromatography-Tandem Mass Spectrometry Coupled with Modified QuEChERS Procedure

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Abstract: Five plant growth regulators, uniconazole, 6-benzyladenine, kinetin, 4-chlorophenoxyacetic acid and diethyl aminoethyl hexanoate, frequently used in apple, grape, strawberry, peach and orange, were determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). A modified QuEChERS (Quick, easy, cheap, effective, rugged and safe) procedure was used in sample pretreatment, which involved extraction with acetonitrile containing 1% acetic acid and clean-up with a mixture of ODS and $MgSO_4$. Mass spectrometric determination was performed in multiple reaction monitoring mode (MRM) using external standards for calibration. The results indicated that the calibration curves for the target analytes were linear in the range of 1–100 μg L^{-1} with correlation coefficients greater than 0.999. The recoveries at three spiked levels ranged from 71.82% to 109.24% with RSDs from 0.57% to 12.43%. The respective limits of detection (LOD) and limits of quantification (LOQ) were 0.15–0.45 μg kg^{-1} and 0.51–1.50 μg kg^{-1} for uniconazole, 0.28–0.53 μg kg^{-1} and 0.94–1.75 μg kg^{-1} for 6-benzyladenine (6-BA), 0.15–69 μg kg^{-1} and 0.51–2.29 μg kg^{-1} for kinetin (6-KT), 0.22–0.60 μg kg^{-1} and 0.74–2.01 μg kg^{-1} for 4-chlorophenoxyacetic acid (4-CPA), 0.88–1.89 μg kg^{-1} and 2.89–6.29 μg kg^{-1} for diethyl aminoethyl hexanoate (DA-6).

Key Words: Ultra-performance liquid chromatography-tandem mass spectrometry; Plant growth regulators; Fruit

1 Introduction

Plant growth regulators (PGRs), produced naturally by plants or synthesized in the laboratory, are small organic molecules acting inside plant cells, altering the growth and development of plants[1]. The compounds can promote, inhibit or change plant physiological or morphological processes at very low concentrations levels[2]. Uniconazole, 6-benzyladenine (6-BA), kinetin (6-KT), 4-chlorophenoxyacetic acid (4-CPA) and diethyl aminoethyl hexanoate (DA-6) have been widely used in fruit production[3–5]. Uniconazole can enhance stress tolerance in plants by inhibition of gibberellin biosynthesis[6,7]. As artificial cytokinins, 6-BA and 6-KT are involved in the regulation of processes such as cell division, apical dominance and growth of lateral buds, and delay of senescence of plant organs[8–11]. 4-CPA is widely used in cultivation to enhance growth and improve fruit setting and development of fruit[12]. DA-6 has the capacity of stimulating the synthesis of chlorophyll, protein and nucleic acid and promoting the photosynthetic rate and carbon and oxygen metabolism of plants[13].

Although PGRs have been widely used in modern agricultural production, the hazards in relation to food safety and human health have increasingly become the focus of

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world attention[14]. To protect the population against such contamination and the associated adverse health effects, some countries and international organizations, including the United States, European Union and Japan, have promulgated their own maximum residue limits for PGRs in fruit[15–17]. As a consequence, food commodities are subject to rigorous control measures to assure non-violation of the maximum residue limits. Consequently, a sensitive and accurate multi-residue analytical method is essential for monitoring and controlling the residue levels of PGRs at low concentration levels[18]. Till now, various methods have been reported for the determination of PGRs in plants, such as surface-enhanced Raman spectroscopy (SERS)[19], *L*-Phe fluorescence quenching[20], ionic liquid/salt aqueous two-phase system (IL-ATPS) and ionic liquid based microwave-assisted extraction procedure (IL-MAE)[21], high-performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS) and capillary electrophoresis[22-24]. However, these afore-mentioned methods have specific limitations for PGR determination, such as low selectivity and sensitivity, or poor applicability[25]. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been applied to the determination of PGRs in recent years because of its high sensitivity[13,26–28]. Due to their cumbersome sample preparation processes, however, these methods cannot meet the requirement of accurate, economical and efficient measurement. Recently, sample pretreatment with QuEChERS (Quick, easy, cheap, effective, rugged and safe) method was introduced and attracted much attention due to its advantages such as acceptable recoveries for acidic, neutral and basic pesticides, short extraction times and low consumption of organic solvents[29–31]. To the best of our knowledge, there has been no report on simultaneous determination of the five PGRs mentioned above in fruits.

In this research, apple, peach, orange, grape and strawberry were investigated as typical categories of pome, stone, citrus, berry fruit and aggregate fruit. A simple, rapid and sensitive method based on a modified QuEChERS procedure was developed for simultaneous determination of uniconazole, 6-BA, 6-KT, 4-CPA and DA-6 by UPLC-MS/MS. The results indicated that the method was efficient, sensitive and accurate, and could be applied to the determination of real samples.

2 Experimental

2.1 Instruments and regents

Xevo TQ UPLC-MS/MS (Waters, USA), CF16RXⅡ high speed refrigerated centrifuge (Hitachi, Japan), and Milli-Q Synthesis Ultrapure Water System (Millipore, USA) were used in the experiment.

Acetonitrile and ammonium acetate were purchased from Thermo Fisher Scientific (USA). Acetic acid was purchased from J.T. Baker (USA). ODS (50 μm, 60 Å) and PSA (40–60 μm) were obtained from Agela Technology Co. Ltd (Tianjin, China). Anhydrous $MgSO_4$ was purchased from Fengchuan Chemical Reagent Technology Co. Ltd (Tianjin, China). Sodium chloride was purchased from Kermel Chemical Reagent Technology Co. Ltd (Tianjin, China), dried at 140 °C for 4–5 h. Uniconazole, 6-BA, 6-KT and 4-CPA were obtained from Sigma Aldrich (USA). DA-6 was purchased from Shanghai Pesticide Research Institute (Shanghai, China). The fruit samples were purchased from local markets.

2.2 Mass spectrometry conditions

The instrument featured an electrospray (ESI) source and was operated in both positive and negative ion modes. Quantitation was performed using multiple reaction monitoring (MRM) mode. The source temperature was 150 °C and the desolvation gas temperature was 350 °C. The desolvation gas and nebulizer gas (N_2) were set at 650 L h^{-1} and 50 L h^{-1}, respectively. The flow rate of the collision gas (Ar) was 0.14 mL min^{-1}.

2.3 Chromatographic conditions

Chromatography was performed on a reversed phase HSS T3 column (2.1 mm × 100 mm, 1.8 μm) from Waters Scientific (USA). Mobile phase A and B were acetonitrile and water respectively. The linear gradient program is as follows: 0–2 min, 10%–90% A; 2–4 min, 90%–10% A; 4–5.5 min, 10%–90% A. The injection volume was 5 μL with a flow rate of 0.4 mL min^{-1} and the column temperature was maintained at 40 °C.

2.4 Preparation of sample solution

Fruit samples were homogenized with a homogenizer. The extraction and clean-up steps of the QuEChERS method were modified and performed as follows: Firstly, 10 g of homogeneous sample was added to 10 mL of acetonitrile with 1% (*V/V*) acetic acid in a 50-mL centrifuge tube, which was then shaken for 2 min. Next, 2 g of NaCl and 4 g of anhydrous $MgSO_4$ were added and the mixture was immediately shaken for a further 2 min. The solution was centrifuged at 9,000 rpm for 3 min. Then 1 mL of the acetonitrile layer was transferred to a 2-mL centrifuge tube containing 25 mg of ODS and 75 mg of $MgSO_4$. The supernatant was filtered through a0.22-μm membrane filter and transferred to an autosampler vial for UPLC-MS/MS analysis.

2.5 Preparation of standards

Five primary stock solutions of uniconazole, 6-BA, 6-KT,