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Original Article

Modified QuEChERS method for 24 plant growth regulators in grapes using LC-MS/MS

Q4 *Chieh-Han Pu, Shao-Kai Lin, Wei-Chen Chuang, Tsyr-Horng Shyu* *

Division of Residue Control, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Taichung, Taiwan

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ABSTRACT

A multiresidue analytical method was developed for grapes for the following 24 plant growth regulators: 1-naphthylacetamide, 2,3,5-triiodobenzoic acid, 2,4,5-T, 2-naphthoxyacetic acid, 3-indolylacetic acid, 4-(3-indolyl)-butyric acid, 4-chlorophenoxyacetic acid, 4-nitrophenol, 6-benzylaminopurine, N6-isopentenyladenine, butralin, chlormequat chloride, chlorphonim-Cl, cloprop, forchlorfenuron, gibberellic acid 3, gibberellic acid 4, gibberellic acid 7, inabenfide, mepiquat chloride, paclobutrazol, prohydrojasmon, thidiazuron and uniconizole-P. The compounds were extracted from grape samples using an extraction method modified from the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. Liquid chromatography – tandem mass spectrometry was used for the detection and quantification of the compounds. Validation of the method was performed by using recovery studies at both intra-day and inter-day intervals, as well as by evaluation of the matrix effect, limit of quantification, trueness and precision. We used matrix-matched calibrations for the quantification of the compounds, which all resulted in determination coefficients (r^2) higher than 0.995. The limit of quantification ranged from 0.1 to 5 ng/mL. Recovery studies using three spiking concentrations at varying levels showed recoveries of 70.2–112.6% and 67.5–101.8% at intra-day and inter-day intervals, respectively. Relative standard deviations were below 20% for the recovery studies. The extraction method were further validated by performing recovery study and matrix effect test in six different grape varieties from Taiwan and the United States and all resulted in comparable results. Application of the established method to 50 grape samples, resulted in the detection of chlormequat chloride and forchlorfenuron residues in the tested grapes. The results of the method validation and real sample analysis shows the extraction method is therefore suitable for routine monitoring of residue in grapes.

1. Introduction

Plant growth regulators (PGRs) are natural or synthetic chemical compounds that regulate plant physiologies at minimal amounts. PGRs have been widely used in agricultural practices, such as grape cultivation, to achieve desirable traits for high quality and production. Studies on grape cultivation have indicated that regular usage of gibberellins

* *Corresponding author*. Fax: +886 4 23324738.
E-mail address: ths@tactri.gov.tw (T.-H. Shyu).

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and cytokinins promote floral cluster elongation [1–3]. The use of abscisic acid has also been found to improve grape color [2].

PGRs have specific functions and can mainly be classified into auxins, cytokinins, gibberellins and inhibitors [4,5]. Auxin indole compounds such as 3-indolylacetic acid (IAA), 4-(3-indolyl)-butyric acid (IBA), 2-naphthoxyacetic acid (2-NOA), 1-naphthylacetamide (1-NAD), atonik, 4-chlorophenoxyacetic acid (4-CPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) can be used in fruits to promote cell enlargement and differentiation, root formation and fruit enlargement [4,6]. Cytokinins such as N6-isopentenyladenine (2iP), forchlorfenuron (CPPU), 6-benzylaminopurine (6-BAP) and thidiazuron (TDZ) are N6-substituted adenine derivatives that stimulate cell division and growth [6,7]. Gibberellic acids (GAs) such as GA3, GA4, and GA7 are terpenoids that promote seed dormancy breakage and flower induction; studies have shown that GAs are used to promote cluster loosening in seedless grapes [5,6,8]. Inhibitors of GA biosynthesis include chlorphonium chloride, chlormequat chloride (CCC), mepiquat chloride, paclobutrazol (PBZ) and uniconazole-P [8,9]. CCC promotes crop production during periods of moisture stress, but can inhibit crop production during periods of drought stress [5]. Auxin transport inhibitors such as 2,3,5-triiodobenzoic acid (TIBA) have been found to affect crop growth, flowering and production [5,10–13].

Nevertheless, the application of chemicals in agricultural practices has led to concerns regarding consumer health and environmental contamination. Studies have shown that CCC may affect mammalian fertility [14] and that GA may increase mast cell recruitment and affect the level of Substance P [15]. An analysis of residues of atonik and 4-nitrophenol in the urine of adults living in the United States had a detection rate and residue mean of 41% and 1.6 ng/mL, respectively [16,17]. Thus, international and national regulatory agencies for pesticide residues such as Codex, as well as from those from Taiwan, the European Union (EU) and the United States (US) have developed PGR maximum residues limits (MRLs) in order to monitor and regulate PGR residues in crops.

Multiresidue analysis methods are commonly used in routine residue monitoring to ensure compliance with MRLs. The development of the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method shortens the multiresidue extraction to less than 2 h, it requires small amounts of solvents, and it uses simple procedures to achieve favorable extraction results [18]. Studies on methods for PGR analysis have focused either on analysis of single compounds such as prohexadione and 6-benzylaminopurine [19,20] or on multiple compounds analysis [21–24]. An analytical method for rapeseed encompassing 12 PGRs using ultrasound-assisted extraction and liquid chromatography – tandem mass spectrometry (LC-MS/MS) was established [23]. The analytical method for rapeseed requires a further re-extraction procedure and rotatory evaporation, which would require more time than would the QuEChERS method. A modified QuEChERS method was developed for 5 and 15 PGRs in bean sprouts, tomatoes, oranges, and peaches [21,24]. The modification methods mainly changed the extraction solvent that was used or excluded the cleanup procedure. Published PGR multiresidue analytical methods either are suitable for a few compounds, require complex procedures, or exclude cleanups to compensate for adequate extraction recoveries. However, complex monitoring analysis procedures have disadvantages due to increased time consumption, and the removal of cleanup procedures may easily lead to instrumental contamination after routine monitoring.

Grapes are a highly preferred fruit in Taiwan: their production and their production value reached 85,434 metric tons and five million New Taiwan dollars, respectively, in 2015. Imported grapes are the seventh highest fruit import products in Taiwan, reaching 57,761 metric tons in 2015 [25]. The production of grapes is known to regularly make use PGRs. However, PGRs residues are not regularly monitored in Taiwan; therefore, PGR usage in grape production and residues in grapes remain unclear. This study aims to develop a modified QuEChERS method for PGRs analysis in grapes that includes various PGR classifications. The established method in this study was then used to analyze 50 grape samples in Taiwan in order to evaluate PGR residues in the grapes.

2. Materials and methods

2.1. Chemicals and standard solutions

Analytical-grade ammonium acetate (98%), magnesium sulfate anhydrous (≧98.0%), trisodium citrate dihydrate (≧99.0%), disodium hydrogen citrate sesquihydrate (≧99.0%), sodium chloride (≧99.5%), formic acid (FA, 98–100%) and HPLC-grade methanol (≧99.8%) were purchased from Merck. Primary secondary amine (PSA) was purchased from Agilent Technologies, and HPLC-grade acetonitrile (≧99.9%) was purchased from J.T.Baker. HPLC-grade acetone (99.98%) was from Burdick & Jackson. Highly purified water (Milli-Q, Millipore) was used in the mobile phase.

The chemical structures of the 24 PGRs are shown in Fig. 1. Certified standards of 1-NAD (99.0%), IAA (99.3%), 4-nitrophenol (99.9%) and 2iP (>90%) were purchased from Sigma–Aldrich/Fluka (St. Lousis, MO, USA). Certified standards of TIBA (99.0%), 2,4,5-T (99.0%), 2-NOA (96.5%), IBA (99.0%), 4-CPA (99.5%), 6-BAP (99.0%), butralin (99.0%), CCC (99.0%), chlorphonim-Cl (99.0%), cloprop (99.0%), CPPU (99.2%), GA3 (98.0%), inabenfide (98.0%), mepiquat chloride (99.0%), PBZ (98.5%) and TDZ (99.0%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Certified standards of GA4 + GA7 (90.0%), prohydrojasmon (99.8%) and uniconizole-P (99.5%) were purchased from Duchefa Biochemie (Haarlem, The Netherlands), Wako (Osaka, Japan) and Chem Service (Pennsylvania, USA), respectively. Standard stock solutions at concentrations of 1000 μg/mL in solvents (mainly methanol, acetone, or acetonitrile) were prepared and stored at −20 °C.

2.2. Mass instrument

Chromatography analysis was performed with an AQUITY UPLC® (Waters, USA). PGRs were separated with a BEH C18 1.7 μm pre-column (AQUITY UPLC® VanGuard™, 2.1 mm diameter, 5 mm length) linked to a BEH C18 1.7 μm column (AQUITY UPLC®, 2.1 mm diameter, 100 mm length). Ammonium acetate (1 mM) dissolved in 0.1% FA solution in H_2O and

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