Food Chemistry 175 (2015) 395–400

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of ametoctradin residue in fruits and vegetables by modified quick, easy, cheap, effective, rugged, and safe method using ultra-performance liquid chromatography/tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 24 November 2014
Accepted 29 November 2014
Available online 5 December 2014

Keywords:
Residue
Ametoctradin
Vegetables
Fruits
UPLC–MS/MS

ABSTRACT

A rapid, effective and sensitive method to quantitatively determine ametoctradin residue in apple, cucumber, cabbage, tomato and grape was developed and validated using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS). The target compound was determined in less than 5.0 min using an electrospray ionisation source in positive mode (ESI+). The limit of detection was below 0.043 μg kg^{-1}, whereas the limits of quantification did not exceed 0.135 μg kg^{-1} in all five matrices. The method showed excellent linearity ($R^2 > 0.9969$) for the target compound. Recovery studies were performed in all matrices at three spiked levels (1, 10 and 100 μg L^{-1}). The mean recoveries from five matrices ranged from 81.81% to 100.1%, with intra-day relative standard deviations (RSDr) in the range of 0.65–7.88% for the test compound. This method will be useful for the quick and routine detection of ametoctradin residues in potato, grape, cucumber, apple and tomato.

1. Introduction

Fungicides are widely used in agriculture to protect crops, fruits and vegetables in the field and during the storage process. Therefore, the concentration of pesticide residues in many products, including fruits and vegetables, must be monitored. Their regulations have been developed (Tian et al., 2012) for food safety. Ametoctradin, 5-ethyl-6-octyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (Fig. 1) belongs to a new class of chemistry called triazolopyrimidylamines in FRAC group 45, which was discovered and developed by BASF in 2010.

It is well known that grape and apple are among the most important horticultural fruit crops in the world. Tomato, cumber and cabbage are widely distributed in the world and considered important vegetables. Unfortunately, all cultivars are susceptible to several diseases; fungi and oomycetes are the major pathogens that compromise the cultivation and economic profit from these plants (Boubakri et al., 2013; Gauthier et al., 2014; Wang et al., 2014). Ametoctradin is a mitochondrial respiration inhibitor that interferes with complex III (complex bc1) in the electron transport chain of the pathogen; thus, ATP synthesis in the fungal cells is inhibited, and ametoctradin controls all major oomycete pathogens, e.g., *Plasmopara viticola* in grapes, *Phytophthora infestans* in potatoes and tomatoes, and *Pseudoperonospora cubensis* in cucurbits (Merk et al., 2011). Some studies have demonstrated that ametoctradin does not show cross-resistance to fungicide classes such as Qo inhibitors, phenylamides, and carboxylic acid amides (Merk et al., 2011). Hence, this fungicide offers a significant improvement for growers in controlling downy mildew and late blight in potato, grape, cucumber, apple, tomato and other crops. In addition, it is in the process of being registered on a global scale for use against diseases of fruit and vegetable crops. For food safety, detailed investigations on its residue and detection are notably important. However, only a few studies on ametoctradin have been reported, and most of them involve bactericidal activity (Brunelli, Portillo, Pirondi, Vignini, & Vigna, 2012; Noguerol-Pato, Torrado-Agrasar, Gonzalez-Barreiro, Cancho-Grande, & Simal-Gandara, 2014; Viglione, Ronco, Freccero, Bigot, & Ferrari, 2014) or the mechanism of action (Merk et al., 2011). At present, there is only 1 analytical study on the determination of ametoctradin residues in grape, pepper, hulled rice and potato using high-performance liquid chromatography (HPLC)-PDA (Do et al., 2013). However, HPLC linked to PDA detection generally lacks the necessary sensitivity and selectivity to measure target compounds. In contrast, UPLC–MS/MS detection is an effective alternative technique that overcomes many inherent shortcomings of the current methods. Unfortunately, there is no available literature on the

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http://dx.doi.org/10.1016/j.foodchem.2014.11.158
0308-8146/

analytical methods to determine ametoctradin residues in potato, grape, cucumber, apple and tomato using UPLC–MS/MS.

Among the existing determination techniques, ultra high-pressure liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) is a notably powerful tool to identify and quantify various thermally stable pesticides in complex environmental matrices, as shown in numerous literature published in recent years for fruits and vegetables (Bakirci, Acay, Bakirci, & Otles, 2014; Du et al., 2013; She et al., 2012; Zhu et al., 2013). In MS/MS, the use of a multiple reaction monitoring (MRM) mode significantly decreases the detection limit because of an increased signal-to-noise ratio. UPLC in combination with tandem MS is a more robust analytical tool for pesticide residue analysis in different matrices (Xu et al., 2012). Sample pretreatment plays an important role in analysing pesticide residues. In 2003, Anastassiades et al. first developed the excellent QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method to monitor pesticides in vegetables and fruits (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003). In a follow-up study, Lehotay et al. confirmed that the QuEChERS method could effectively determine 229 pesticide residues in agricultural samples (Lehotay, Kok, Hiemstra, & Bodegraven, 2005). In recent years, the QuEChERS method has also been widely applied to determine pesticide residue in vegetables and fruits (Ko et al., 2014; Zhang, Zhang, & Jiao, 2014). This method has many advantages over traditional techniques, such as high recovery for a wide polarity and volatility range of pesticides; high sample throughput and the use of smaller amounts of organic solvent (Wu et al., 2014).

To the best of our knowledge, this study is the first time the QuEChERS method was applied to analyse ametoctradin residue in fruits and vegetables using UPLC–MS/MS. In the present paper, a modified QuEChERS method to determine ametoctradin in fruits and vegetables using UPLC–MS/MS was developed and validated. To achieve satisfactory extraction and purification of ametoctradin, different types of sorbents (primary secondary amine (PSA), graphitized carbon black (GCB) and octadecylsilane (C18)) and extraction solvent (acetonitrile and methanol) were investigated. The method was successfully used to analyse ametoctradin in actual fruits and vegetables samples.

2. Experiment section

2.1. Reagents and materials

The analytical standard ametoctradin (purity 99.3%) was obtained from the Institute for the Control of Agrichemicals, Ministry of Agriculture (ICAMA). Chromatography grade acetonitrile was purchased from Sigma–Aldrich (Steinheim, Germany). analytical grade sodium chloride (NaCl), acetic acid and anhydrous magnesium sulfate (anhydrous $MgSO_4$) were purchased from Beijing Chemical Company (Beijing, China). Ultra-pure water was prepared using a Milli-Q reagent water system (Bedford, MA, USA). Primary secondary amine (PSA, 40 μm), graphitized carbon black (GCB) and octadecylsilane (C18) were purchased from Agela Technologies Inc. (Beijing, China).

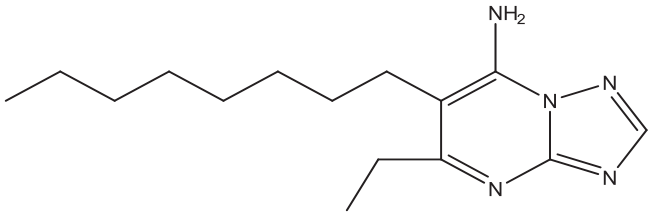

Fig. 1. Chemical structure of ametoctradin.

The standard stock of ametoctradin (100 mg L^{-1}) was prepared in pure acetonitrile. Standard working solutions at 1, 10, 50, 100, 200, and 500 μg L^{-1} were prepared from the stock solution by serial dilution with acetonitrile. Correspondingly, matrix-matched standard solutions were obtained at 1, 10, 50, 100, 200, and 500 μg L^{-1} by adding blank sample extracts (tomato, cabbage, cucumber, grape and apple) to each serially diluter standard solution. All solutions were stored in a refrigerator in the dark at 4 °C, and the working standard solutions underwent no degradation for 3 months. Six blank food matrices were purchased from the supermarket in Beijing, which were pre-checked to confirm the absence of the target compound, and stored in the dark below 4 °C until the analysis.

2.2. Instrumentation

The analyses were performed on a Waters Acquity UPLC system, which included a Waters Acquity UPLC binary solvent manager, an Acquity UPLC manager, and an Acquity column heater equipped with a Waters Acquity UPLC BEH C18 column (2.1 × 100 mm 1.7-μm particle size; Milford, MA, USA). The mobile phase consisted of pure water (A) and chromatography grade acetonitrile. The gradient program was as follows: time 0 min, 90% A; 1.5 min, 10% A; 3 min, 10% A; 3.1 min, 90% A; and 4.0 min, 90% A. The flow rate was 0.3 mL min^{-1} and the injected sample was maintained at 5 μL Separation and stabilisation were achieved in 5 min. The column was maintained at 40 °C for reduce viscosity and the temperature in the sample manager was maintained at 4 °C.

The ametoctradin analysis was conducted on a triple quadrupole (TQD) mass spectrometer (Waters Corp., Milford, MA, USA), which was equipped with an electrospray ionisation (ESI) source. The MassLynx software (version 4.1) was used for instrument control and data acquisition. MS/MS detection was performed in the positive ionisation mode, and the monitoring conditions were optimised for the target compounds. The nebulizer gas was 99.95% nitrogen, and the collision gas was 99.99% argon with a pressure of 2×10^{-3} mbar (2×10^{-5} MPa) in the T-Wave cell. The typical conditions were as follows: the capillary voltage was set at 3.0 kV, whereas the source temperature and desolvation temperature were maintained at 120 and 350 °C, respectively. A cone gas flow of 50 L h^{-1} and desolvation gas flow of 500 L h^{-1} were used. The multiple reaction monitoring (MRM) mode was operated for the target compounds at 160 ms. Under the described conditions, the retention time of ametoctradin was approximately 2.60 min. All parameters for MRM transitions, cone voltage and collision energy were optimised to obtain the highest sensitivity and resolution (Table 1).

2.3. Sample preparation procedure

Apple, cucumber, cabbage, tomato and grape were purchased form the local supermarket. These samples were not applied and contaminated by the target compound. All samples were chopped and homogenised in an Ultra-Turrax homogeniser (IKA-Werke, Staufen, Germany) and stored in the dark at less than −20 °C until the analysis. Ten-gram (accurate 0.01 g) samples were weighed into a 50 mL Teflon centrifuge tube and three concentration levels working standard solution were added. The tube was centrifuged for 30 s and allowed to stand for 1 h at room temperature. Then, 10 mL acetonitrile (containing v/v 0.1% acetic acid) was added to extract the pesticide. The tubes were capped and immediately centrifuged vigorously for 5 min and centrifuged (4000 RCF) for 5 min. Then, 1.5 mL of the upper layer (acetonitrile) was transferred into a 2 mL single-use centrifuge tube, which contained an amount of sorbent (50 mg PSA and 150 mg $MgSO_4$ for cucumber, apple and grape; 50 mg C18 and 150 mg $MgSO_4$ for tomato; 20 mg PSA,