Chemosphere 207 (2018) 329–336

Contents lists available at ScienceDirect

Chemosphere

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

A simple multi-residue method for determination of plant growth retardants in *Ophiopogon japonicus* and soil using ultra-performance liquid chromatography–tandem mass spectrometry

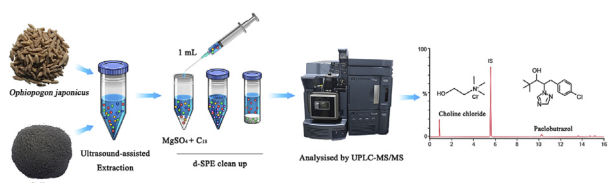
Xiangsheng Zhao [a], Yan Mu [b], Meihua Yang [a, c, *]

[a] *Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Haikou 571100, China*
[b] *Yunnan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Jinghong 666100, China*
[c] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China*

HIGHLIGHTS

- A versatile UPLC-MS/MS analytic method for 11 plant growth retardants is proposed.
- Octadecylsilyl and magnesium sulfate anhydrous were used as the d-SPE sorbent.
- Paclobutrazol and choline chloride were detected at high frequency in *O. japonicus*.
- Residual level of paclobutrazol in *O. japonicus* planted soil was high.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 March 2018
Received in revised form
8 May 2018
Accepted 18 May 2018
Available online 18 May 2018

Handling Editor: Keith Maruya

Keywords:
UPLC-MS/MS
Plant growth retardants
Ophiopogon japonicus
Soil
d-SPE

ABSTRACT

Plant growth retardants may play an important role in regulation of yield and quality of crops, fruits, and vegetables. Such compounds have begun to be used in the cultivation of traditional Chinese medicines (TCMs), especially for root medicines. Although the potential risks to human health of these compounds has attracted increasing attention, analytical methods for detection of plant growth retardants in TCMs remain poorly investigated. In this study, an effective and reliable method for simultaneous determination of 11 plant growth retardants in *Ophiopogon japonicus* and soil samples was developed by ultra-performance liquid chromatography-triple quadrupole tandem mass spectrometry (UPLC-QqQ-MS/MS). Extraction was conducted in acetonitrile containing 1.0% (*v*/*v*) acetic acid with ultrasonication. The octadecylsilyl (C_{18}) and $MgSO_4$ were used as the dispersive-solid phase extraction (d-SPE) sorbent and provided satisfactory recoveries for the analytes. The conditions of extraction and LC-MS/MS were optimized to achieve the highest recovery and sensitivity. Good linearity was achieved within a wide range with all correlation coefficients exceeding 0.9950. The recoveries of all analytes in *O. japonicus* and soil samples ranged from 57.37% (choline chloride) to 99.93% (trinexapac-ethyl) and from 54.37% (daminozide) to 94.82% (triadimenol), respectively. The limits of quantifications ranged from 0.03 to 3.54 μg/L. The proposed method was successfully applied to detect and quantify 11 plant growth retardants in empirical *O. japonicus* and soil samples. High frequency of paclobutrazol and choline chloride was found in *O. japonicus* samples. In addition, paclobutrazol showed a high residual concentration (>1100 μg/kg) in the soil of *O. japonicus* indigenous production.

* Corresponding author. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China.
E-mail address: yangmeihua15@hotmail.com (M. Yang).

https://doi.org/10.1016/j.chemosphere.2018.05.105
0045-6535/

1. Introduction

Ophiopogon japonicus (Thunb.) Ker-Gawl. (Liliaceae) is an evergreen perennial plant that is widely distributed in southern China, especially in Sichuan and Zhejiang provinces. Its root (Maidong in Chinese) has been used in traditional Chinese medicines (TCMs) for more than 2000 years as a tonic agent, and is effective in treating dry cough, hyperactivity, insomnia, cardiopathy, constipation, and diabetes caused by 'internal heat'. Nowadays, it is also an indispensable component in the treatment of coronary atherosclerotic cardiopathy and viral myocarditis in combination with *Panax ginseng* and *Schisandra chinensis* (Chinese Pharmacopeia Commission, 2015; Yan et al., 2016a and Yan et al., 2016b). In addition to its medicinal value, *O. japonicus* is also widely used in the general diet and listed as an edible Chinese medicine by the Chinese Ministry of Public Health (Chen et al., 2016). The current supply of *O. japonicus* to markets is mainly dependent on field cultivation. To meet market demand for *O. japonicus*, the use of plant growth regulators (PGRs) is an effective means of increasing yield.

Plant growth regulators (PGRs), or exogenous plant hormones, are a class of synthetic compounds that show similar physiological activity as endogenous plant hormones (Lu et al., 2014). The PGRs have the capacity to promote, inhibit or modify plant physiological or morphological processes at very low concentrations. The PGR compounds can be divided into growth promoters and growth retardants based on the modes of action (Giannakoula et al., 2012). Plant growth retardants are widely used in crops, fruits and vegetables to enhance shoot multiplication, rooting or survival rates during the acclimatization stage. Plant growth retardants can be mainly classified into quaternary ammonium compounds (e.g., chlormequat chloride, mepiquat chloride, and choline chloride), nitrogen-containing heterocyclic compounds (e.g., paclobutrazol, uniconazole, and inabenfide) and other compounds (e.g., daminozide and trinexapac-ethyl) (Kim et al., 2003; Zhai et al., 2013). Owing to their specific properties, plant growth retardants are widely used in the cultivation of medicinal plants and especially for root herbs (Zhai et al., 2013). However, in recent years indiscriminate and excessive use of plant growth retardants have affected the quality of TCMs and are threatening public health, causing disorders such as dysfunction of the immune system and hematopoietic system, digestive and endocrine disorders, chronic kidney diseases, cancer, and other potential diseases (Cao et al., 2017). In addition, an inherent impact associated with the use of some plant growth retardants is long-lasting residual activity in agricultural soils, which seriously inhibits subsequent crop growth (Kim et al., 2003). To protect public health and regulate the application of PGRs, many countries have set maximum residue limits (MRLs) in crops, fruits and vegetables. The European Union set a MRL of 0.5 mg/kg for paclobutrazol in citrus fruit (European Commission, 2005). The USA established MRLs of 1.0 and 0.2 mg/kg of triadimefon and triadimenol in apple and banana, respectively (US Environmental Protection Agency Office of Pesticide Programs, 2009). The MRL for trinexapac-ethyl in crops is 0.6 mg/kg as regulated by the Ministry of Health, Labour and Welfare of Japan (Ministry of Health, Labour and Welfare, Japan. http://www.m5.ws001.squarestart.ne.jp/foundation/search.html). In China, the MRLs of seven plant growth retardants (paclobutrazol, chlormequat, triadimenol, triadimefon, maleic hydrazide, daminozide and uniconazole) in crops was set according to GB 2763-2016 (GB 2763-2016, China, 2016). However, plant growth retardants residues in TCMs are not regularly monitored in China and other countries. In a previous survey, we found that different types of plant growth retardants (especially paclobutrazol and choline chloride) were widely used in the cultivation of *O. japonicus*. In addition to the harmful effects of paclobutrazol on human and animal health, the content of total saponins in *O. japonicus* was distinctly reduced after spray application of the compound (Wei et al., 2016). To date, limited information is available on the use of plant growth retardants in *O. japonicus* production, and their residues in *O. japonicus* tissues and the soil remain unclear. Therefore, in view of their potentially harmful effects, it is vital to develop an accurate, convenient, sensitive, quantitative method for analytically monitoring the proper use of plant growth retardants.

Analysis of plant growth retardants is challenging as they are often present at a low concentration in complex matrices, for example in TCMs. A variety of analytical methods have been developed to estimate PGR concentrations in plants, such as gas chromatography-mass spectrometry (GC-MS) (Wu et al., 2014), gas chromatography-tandem mass spectrometry (MS/MS) (Xu et al., 2014), capillary electrophoresis-mass spectrometry (CE-MS) (Juan-García et al., 2014), high-performance liquid chromatography (HPLC) coupled with different detectors including a fluorescence detector (FLD) (Li et al., 2015), diode array detector (DAD) (Das and Prasad, 2015), MS (Mao et al., 2014), or MS/MS (Cai et al., 2015), and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Liu et al., 2017). Most plant growth retardants are polar molecules, and are hard to gasified and easily decomposed while heated. Thus, the analysis of plant growth retardants using GC is generally difficult. Although some PGRs can be analyzed by GC after derivatization, the derivatization process is troublesome and time-consuming, which limits its utility for a large number of samples (Cao et al., 2017). Because the matrix of TCMs is complex, the application of CE-MS technology for PGR residues analysis is difficult (Wang et al., 2016). UPLC-MS/MS is the most widely used analytical method for a complex matrix (Zhou et al., 2015; Ma et al., 2013; Wei et al., 2017), and it can reduce the disadvantages of interference and poor resolution especially using a multiple reaction monitoring (MRM) mode with high selectivity and sensitivity (Zhou et al., 2015).

Little information on simultaneous analysis of multiple classes of plant growth retardants in *O. japonicus* and soil samples is available, the aim of the present work was to develop a sensitive and rapid UPLC-MS/MS method coupled with a dispersive-solid phase extraction (d-SPE) clean-up procedure for simultaneous analysis of multi-class plant growth retardants (choline chloride, chlormequat chloride, mepiquat chloride, daminozide, trinexapac-ethyl, inabenfide, paclobutrazol, triadimefon, triadimenol, uniconazole, and triapenthenol) (Fig. S1) in *O. japonicus* and soil samples. The main factors that influence extraction and clean-up efficiency, including acid contents, volume of the extraction solvent, extraction time, and type and amount of the sorbent, were investigated. As validation, the established method was used to analyze 11 plant growth retardants in 22 batches of *O. japonicus* and 6 soil samples.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade acetonitrile, methanol, formic acid, and acetic acid were purchased from Thermo Fisher Scientific (Fisher, Fair Lawn, NJ, USA). Water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). Plant growth retardant standards, including choline chloride (CC), chlormequat chloride (CCC), mepiquat chloride (Pix), daminozide (B9), trinexapac-ethyl (TNE), inabenfide (IBF), paclobutrazol (PBZ), triadimefon (TDF), triadimenol (TDN), uniconazole (UNI), triapenthenol (TPE), and imidacloprid, were purchased from the Agriculture Environment Protection Institute (Tianjin, China), with purity exceeding 99.0%.