Journal of Chromatography A, 1367 (2014) 39–47

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Using pollen grains as novel hydrophilic solid-phase extraction sorbents for the simultaneous determination of 16 plant growth regulators

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ARTICLE INFO

Article history:
Received 30 August 2014
Received in revised form
25 September 2014
Accepted 25 September 2014
Available online 5 October 2014

Keywords:
Pollen grains
Plant growth regulators
Hydrophilic solid-phase extraction
UHPLC-ESI-MS/MS

ABSTRACT

In this article, pollen grains were for the first time used as a hydrophilic solid-phase extraction (HILIC-SPE) sorbent for the determination of 16 plant growth regulators (PGRs) in fruits and vegetables. Fourier transform infrared spectroscopy (FT-IR), scanning electronic microscopy (SEM) and nitrogen sorption porosimetry (NSP) were used to investigate the chemical structure and the surface properties of the pollen grains. Pollen grains exhibited an excellent adsorption capacity for some polar compounds due to their particular functional groups. Several parameters influencing extraction performance were investigated. A green and simple HILIC-SPE-method using pollen grain cartridge for purification of fruit and vegetable extractions, followed by ultra-high performance liquid chromatography-triple quadrupole tandem mass spectrometry (UHPLC-MS/MS) was established. Good linear relationships were obtained for 16 PGRs with correlation coefficients (*R*) above 0.9980. The limits of detection (LODs) of 16 PGRs in cucumber were in the range of 0.01–1.10 $\mu g \cdot kg^{-1}$. Reproducibility of the method was evaluated by intra-day and inter-day precisions with relative standard deviations (RSDs), which were less than 14.4%. We successfully applied this methodology to analyze 16 PGRs in 8 different kinds of fruits and vegetables. The recoveries from samples spiked with 16 PGRs were from 80.5% to 119.2%, with relative standard deviations less than 15.0%.

1. Introduction

Plant growth regulators (PGRs), also called exogenous plant hormones, are a class of synthetic pesticides, which have a similar physiological activity to their natural hormone analogs [1]. They are able in trace quantities to trigger a variety of basic physiological processes including cell division, enlargement and differentiation, organogenesis, seed dormancy and germination, leaf senescence, and abscission, as well as defense against abiotic and biotic stresses [2]. In recent years, the incidence of overuse of PGRs in fruits and vegetables has gradually been increasing, which has brought some adverse effects to agricultural production. The hazardous effects of PGR residues for both food safety and human health have increasingly become the focus of attention worldwide [3]. Therefore it is necessary to monitor PGR residues.

Due to structural differences, various methods for the determination of PGR residues in fruits and vegetable have focused on the analysis of one analyte [4–8], or of one [9–11] or a few classes [12–14] of PGRs. Ma et al. [2] used mixed solvents to extract 15 PGRs from vegetables, then used this extraction for analysis without further cleanup because none of the sorbents (Florisil, neutral alumina, GCB, and PSA) were suitable for multiresidue analysis purification. Impurities extracted from fruits and vegetables will not only bring about a matrix effect but will also damage analytical instruments in the long run. Therefore, it is necessary to find a sorbent for both extraction and purification to simultaneously quantify different classes of PGRs.

Currently, green analytical chemistry has garnered a great deal of interest among chemists because of its eco-friendly chemical activity [15]. The application of less toxic, preferably natural, reagents during analysis is one of the recent trends in green analytical chemistry [16].

Pollen grains are naturally occurring substances from plants and are the physiological containers that produce the male gametes of seed plants. Exine, the outer pollen wall, consists of an extremely

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http://dx.doi.org/10.1016/j.chroma.2014.09.071
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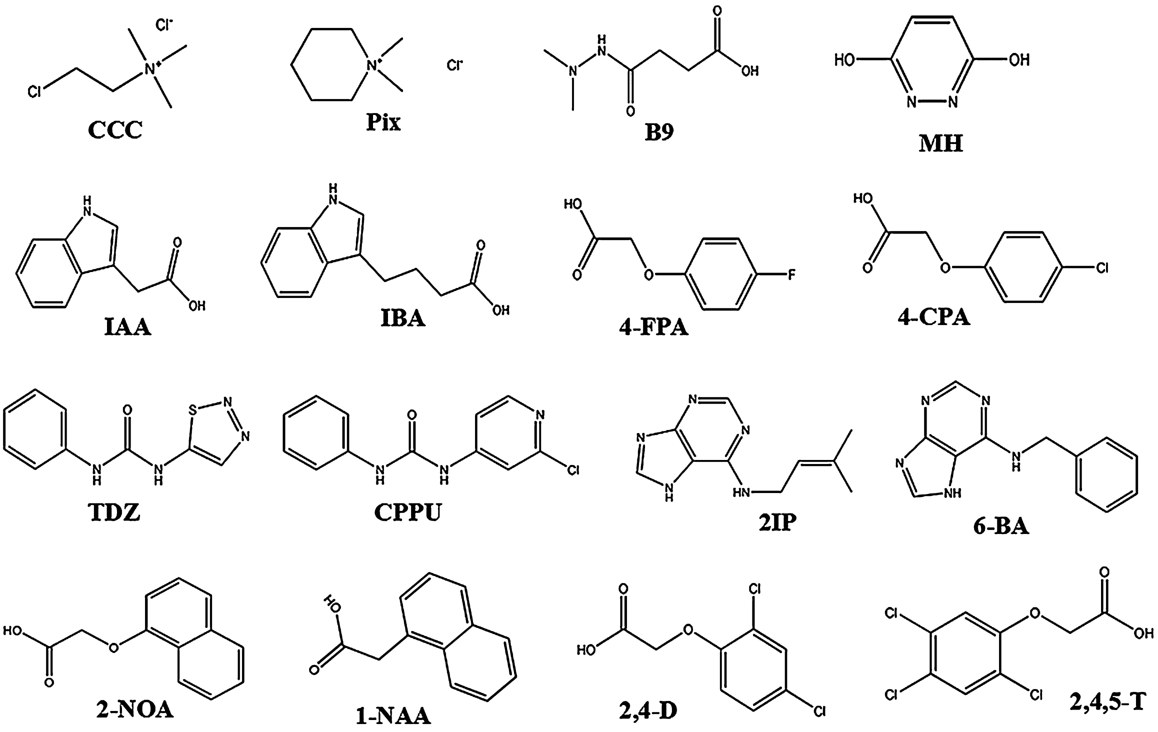

Fig. 1. The chemical structures of 16 PGRs.

stable and complex biopolymer known as sporopollenin, which is highly resistant to chemical attacks and high temperatures [17]. Because of the diverse chemical composition of exine, pollen grains have been used in drug deliveries [18,19], in novel materials as templates [20,21], as carbon microspheres [22], in protein chromatography [23] and during removal of organic pollutants [24]. However, as far as we know, pollen grains have not been used as solid-phase extraction (SPE) sorbents.

SPE is an excellent tool for sample pretreatment because it demands less organic solvents and can remove matrix interferences and pre-concentrate target analytes simultaneously [25–27]. Generally, sorbent materials are a key factor in SPE. Conventional SPE materials such as alkyl-bonded silica are not suitable for the extraction of polar analytes. Therefore, there is considerable interest in developing new selective sorbents for extracting and isolating polar components from complicated matrices [28]. Recently, Cai et al. [29] used a silica SPE cartridge to enrich and purify cytokinins from plant samples based on a hydrophilic interaction.

In this article, pine pollen (*Pinus massoniana* Lamb.) was used as the HILIC-SPE sorbent. Compared with bee pollen, pine pollen is manually collected; hence it has a single flower source, a pure quality, a stable composition, as well as a lack of pesticide residues and animal hormones. This species is typically wind pollinated, having pollen with a small grain size (30–40 μm), a light weight, and abundant quantities (and therefore inexpensive), which make it suitable as an SPE sorbent. Similar to silica, the outer wall of pollen grains have many hydroxyl groups and are chemically stable, so pine pollen grains have the potential to be a promising new HILIC-SPE sorbent. A green and simple approach to extract 16 kinds of hydrophilic PGRs (Fig. 1) was developed. When we activated the pollen SPE cartridge with ACN/H_2O (90/10, v/v), a water-enriched layer onto the pollen grain sorbents was formed. Because of the different distribution ratio of PGRs and interferents in the water-enriched layer onto pollen grain sorbents and solvent (ACN), most PGRs are retained in the water-enriched layer and most interferents flow out with ACN. PGRs were subsequently detected by ultra-high performance liquid chromatography-triple quadrupole tandem mass spectrometry (UHPLC-MS/MS). After optimizing extraction conditions, satisfactory detection limits and analysis precisions were obtained.

2. Methods

2.1. Reagents and materials

Pine pollen (*Pinus Massoniana* Lamb.) was obtained from Anhui Guzhitang Edible Fungus Co., Ltd. (Anhui, China).

PGR standards of forchlorfenuron (CPPU), thidiazuron (TDZ), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-fluorophenoxyacetic acid (4-FPA), 4-chlorophenoxyacetic acid (4-CPA), 2-naphthoxyacetic acid (2-NOA), 1-naphthaleneacetic acid (1-NAA), N6-(2-isopentenyl)-adenine (2IP), 6-benzylaminopurine (6-BA), daminozide (B9), maleic hydrazide (MH), chlormequat chloride (CCC), mepiquat chloride (Pix) and stable isotope-labeled internal standards (IS) d^2-IAA, d^7-6-BA were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China).

Acetic acid, sodium acetate, sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$), formic acid (FA), methanol (MeOH), and acetonitrile (ACN) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). ACN (HPLC grade) was obtained from Tedia Company Inc. (Fairfield, OH, USA). FA (LCMS grade) was supplied by Fluka (Steinheim, Germany). Purified water was obtained using a Millipore Milli-Q apparatus (Bedford, MA, USA).

2.2. Instrumentation

SPE was performed on a Supelco 12-port model SPE Vacuum Manifold (Bellefonte, PA, USA). The Fourier transform infrared (FT-IR) spectrum was determined by a Thermo Nicolet AVTAR-360 (Madison, WI, USA). The microscopic morphology of the pollen grains was characterized using a Quanta 200 scanning electron microscope (SEM, FEI, The Netherland). The surface area was measured on a specific surface area analyzer (Beijing JWGB Sci. &