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Graphene oxide-SiO_2 nanocomposite as the adsorbent for extraction and preconcentration of plant hormones for HPLC analysis

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ABSTRACT

In this research, a modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method based on graphene oxide@SiO_2(SiO_2@GO) nanocomposite as adsorbent of dispersive solid-phase extraction (dSPE) combined with high performance liquid chromatography (HPLC) for the analysis of four plant hormones in different plants was established. The as-prepared SiO_2@GO was characterized by scanning electron microscopy, transmission electron microscopy and infrared spectroscopy. The experimental conditions for dSPE, including the ratio of material to liquid, pH of sample, adsorption and desorption time, desorption temperature as well as desorption solution, were investigated. The detection limits for the analysis of indole-3-acetic acid, indole-3-butyric acid, 1-naphthylacetic acid and abscisic acid were achieved below 0.05 μg mL^{-1}. The established method was applied to the analysis of the plant hormones in fruits, vegetables and other food samples. The obtained results indicated that the method was sensitive, accurate, convenient and quick, which provided an alternative analytical approach for plant hormones in complex matrices.

1. Introduction

Plant hormones as a series of trace organic compounds produced within the plant, play a very important role in controlling plant growth and development [1,2]. They serve as mediums of endogenous plant development and integrate extracellular signals to regulate and optimize plant growth and performance with extremely low concentrations [3,4]. According to their structure and physiological function, the plant hormones can be divided into several major classes including auxins, cytokinins (CK), gibberellins (GA), abscisic acid (ABA), ploy amines, ethylene, jasmonates, salicylic acid and brassinosteroids [5–7]. The plant hormones such as auxins have different specific and vital functions in plant metabolism, which influence apical dominance, cell differentiation and tropism in plants [8]. Generally, auxins consist of indole, naphthalene, chlorinated benzene compounds and their derivatives such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 1-naphthylacetic acid (NAA) and so on [9,10]. Whereas auxins stimulates growing processes such as cell elongation and division, ABA controls plant senescence and responses to stress [11,12]. In order to study the functions of plant hormones, it is necessary to develop a simple, rapid and sensitive method for the determination of plant hormones.

Due to extremely low concentration and serious interference from matrices of various plant tissues, qualitative and quantitative analysis of plant hormones is very difficult. Gas chromatography-mass spectrometry (GC–MS) has been widely used in the analysis of plant hormones. However, time consuming derivatization is usually required since most of plant hormones are nonvolatile compounds. Birkemeyer reported selection of the *N*-methyl-*N*-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) reagent as the most comprehensive derivatization for GC–MS-based multi-targeted profiling of the major phytohormones [13]. In recently, Porfírio developed a method based on dispersive liquid–liquid microextraction (DLLME)/microwave derivatization and GC–MS for quantification of free auxins in semi-hardwood plant cuttings and microshoots [14]. Due to high separation efficiency and small amount sample consumption, capillary electrophoresis (CE) [15] and capillary electrochromatography (CEC) [16,17] were applied to the analysis of plant hormones. However, CE and CEC were not widely used because of poor repeatability and low detection sensitivity. In the past decade, high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) attracted considerable attention in the analysis of plant hormones since high sensitivity compared with LC-UV and not required derivitization compared with GC–MS [18]. Due to high cost of purchase and main-

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tenance, LC–MS was not widely used in small institutes. LC-UV/DAD was considered as good choice for plant hormones analysis because of low cost and easy maintenance. To overcome the main drawback of low detection sensitivity, sample preparation with high efficiency was usually required for LC-UV/DAD.

For the analysis of trace plant hormones, sample pretreatment is the most critical and time-consuming procedure. To cleanup and preconcentration of phytohormones in plants, various techniques have been developed, such as solid-phase extraction (SPE) [19–21], magnetic solid phase extraction (MSPE) [22], solid-phase microextraction (SPME) [23,24], molecularly imprinted solid-phase extraction (MIE) [25], liquid-phase extraction [26], liquid-liquid-solid microextraction [27], single-drop liquid-liquid–liquid microextraction [28], vapor-phase extraction [29], and dispersive liquid–liquid microextraction (DLLME) [30,31]. Nowadays, a method named Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) has been used in the extraction and purification of phytohormones [32].

Graphene has become one of the most exciting research topics because of its outstanding material, physical, and chemical properties since it was first introduced in 2004 [33]. Graphene was considered as an excellent adsorbent because of its electron-rich, π-π electrostatic property and hydrophobic nanomaterial with large specific area [34]. Due to the large delocalized π-electron system, graphene can form a strong π-stacking interaction with the compounds that contained benzene ring [35,36]. However, the escaping from SPE cartridge and irreversible aggregation of graphene could result in reducing the sorption capacity and extraction efficiency of sorbent when being directly used as SPE sorbent [37,38]. In order to solve the problem, one approach was immobilized graphene oxide (GO) on the surface of silica.

Graphene or grapheme oxide based QuEChERS method earned more and more attention in recent years. Guan prepared amine modified grapheme as reversed-dispersive SPE (dSPE) materials combined with LC–MS for pesticide multi-residue analysis in oil crops [39]. Wen developed graphene oxide-based QuEChERS method for extraction of non-steroidal estrogens from water samples [40]. Luo used magnetic grapheme as modified QuEChERS adsorbent for the determination of organochlorine pesticide residues in tobacco [41]. Chen prepared size-controlled magnetite nanoparticles with a graphene and polymeric ionic liquid coating and used as modified QuEChERS adsorbent for the determination of preservatives in vegetables [42]. Up to now, Graphene or grapheme oxide based QuEChERS method has not been applied to analysis of phytohormones.

In the present study, a modified QuEChERS method based on SiO_2@GO nanocomposite as the adsorbent of dSPE combined with HPLC was developed for the analysis of plant hormones (structures see Fig. 1). The proposed method combined the advantages of large surface area of graphene nanomaterials and high extraction efficiency of dSPE, which provide a good choice for the analysis of plant hormones by HPLC technique with satisfied detection sensitivity and low cost. The established method was successfully applied to the analysis of IAA, IBA, NAA and ABA in plants and foods.

2. Materials and methods

2.1. Reagents and apparatus

Graphite powder, tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), ammonia solution ($NH_3{\cdot}H_2O$), hydrogen peroxide solution (H_2O_2), toluene, acetic acid (HPLC grade), ammonium acetate (HPLC grade) and indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 1-naphthylacetic acid (NAA) and abscisic acid (ABA) were purchased from Aladdin (Shanghai, China). Sulfuric acid and hydrochloric acid were provided by Pingmei Chemical Company (Henan, China). Dehydrated alcohol (EtOH) was purchased from Anhui antell food co., Ltd. (Anhui, China). Sodium permanganate and sodium nitrate were supplied by Guangda equipment co., Ltd. (Henan, China). Methyl alcohol (MeOH), acetonitrile (ACN), hexane and acetone with HPLC grade were obtained from Tedia of America. Water used throughout the work was produced by a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA), the wild-type Arabidopsis samples were provided by prof. Bai from state key laboratory of cotton biology (Henan University). Tomato samples were cultivated by our laboratory and other samples were purchased from local supermarket.

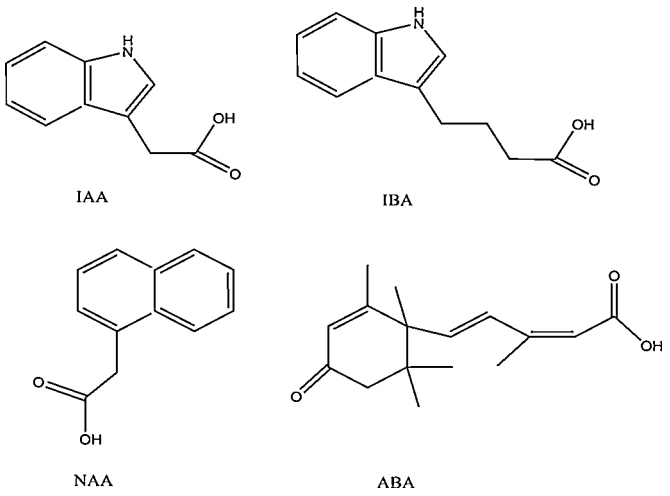

Fig. 1. Structures of target phytohormones.

An Agilent-1260 HPLC instrument was provided by Agilent (Palo Alto, USA), Eclipse XDB-C8 (4.6 mm × 250 mm id, 5 μm particle size) reversed phase column, Transmission electron microscopy (TEM) images were obtained by a JEM-2010 electron microscope (JEOL, Ltd., Japan) operating under 100 kV accelerating voltage. Scanning electron microscopy (SEM) images were conducted by a Nova NanoSEM450 instrument (FEI, USA), fourier-transform infrared spectroscopy (FT-IR) analysis was done on an AVATAR360 (Thermo Fisher, USA).

2.2. Preparation of nanocomposite

2.2.1. Synthesis of GO dispersion

GO was synthesized by Hummers method [43] with some modification through oxidation of graphite and the stepwise preparation was given as follows: graphite flakes (1 g) and $NaNO_3$ (0.5 g) were mixed in 23 mL of H_2SO_4 (98%) in round bottom flasks under ice bath (0–5 °C) with continuous stirring for 30 min, 6 g of potassium permanganate was slowly added into the suspension with several times to keep the reaction temperature below 10 °C. Then, removed the ice bath and the mixture was stirred at 35 °C for 12 h. After that, it was diluted with slow addition of 150 mL water (divided into six times add). The solution was finally added with H_2O_2 to terminate the reaction until the color of the mixture changed into brilliant yellow. The above mixture was centrifuged, and the precipitate was washed with water and 5% (v/v) HCl for several times, and then washed with water until the pH of the solution was neutral. Finally, the GO dispersion was gained by ultrasonic stripping.

2.2.2. Synthesis of SiO_2-NH_2 particles

The SiO_2 microspheres were prepared according to the classic Stöber process [44]. To a flask, 50 mL EtOH and 8 mL ammonia