

Magnetic Solid-Phase Extraction and in Situ Derivatization for Determining Phytohormones and in Oilseeds by Ultra-Performance Liquid Chromatography-tandem Mass Spectrometry

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Research

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Abstract

Background: Phytohormones are a group of naturally occurring signaling molecules which influence physiological processes of oil crops. Simultaneous determination of multiple phytohormones in oilseeds is still a challenge due to their trace concentrations, species diversity, and lipid interference. Therefore, a simple and selective method for the simultaneous determination of multiple phytohormones in oilseeds is urgently needed.

Results: In this study, the $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ nanoparticles were successfully synthesized and used for the first time as an adsorbent for the magnetic dispersive solid-phase extraction of phytohormones from oilseeds. The magnetic dispersive solid-phase extraction and *in situ* derivatization by the addition of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) were ingeniously combined. This efficient pre-treatment method integrated the extraction, purification, and derivatization processes into one single step. Several parameters affecting the efficiency of extraction and derivatization were evaluated.

Under the optimized analysis conditions, satisfactory methodological performance was achieved. High linearities ($R^2 > 0.9928$) at three spiked levels, as well as the low matrix effect (ranged from 16.63 % to 17.06 %) and limits of detection (0.89-13.62 pg/mL) were also obtained. The intra and inter-day relative standard deviations (RSDs) were less than 13.7 % and 11.6 %, respectively. The recoveries were ranged from 80.4 % to 115.1 %. This method was successfully employed for analyzing 12 phytohormones in different oilseeds samples.

Conclusion: A simple and sensitive method based on the magnetic solid phase extraction integrated with *in situ* derivatizations for the profiling of 12 phytohormones, including 9 gibberellins (GAs), indole-3-acetic acid (IAA), abscisic acid (ABA), and jasmonic acid (JA) in a single rapeseed seed was developed by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

Background

Phytohormones are naturally occurring signaling molecules attributing to the regulation of plant growth and development [1]. These molecules are present at trace amounts in plant tissues, and their regulatory mechanisms frequently rely on the complex crosstalk networks among different classes of phytohormones [2, 3]. The study of multiple phytohormones such as Gibberellins (GAs), indole-3-acetic acid (IAA), abscisic acid (ABA), and jasmonic acid (JA) is closely related to agricultural production and the green revolution technology [4]. Phytohormones are usually present at extremely low concentrations, instability, and spatio-temporal distribution in certain plant organs [5, 6]. To elucidate the regulatory mechanisms of phytohormones on plant growth and development, highly sensitive profiling phytohormones in specific organs such as seed has received persistent attention.

Oil crops contribute a total of 93% to the vegetable oils for human consumption worldwide [7]. Vegetable oils are the foremost among all oil products exhibiting specific beneficial and functional properties towards human [8]. In oil crops, accumulating research reported that the phytohormones exhibit a significant effect on seed germination, seedling growth, and yield [9-11], thus the quantification of the phytohormones is critical for the underlying regulation mechanism. However, the analysis of phytohormones in oilseeds matrices is yet a challenging issue, because of the inherent complexity of the matrix. Lipid matrix interferences in oilseeds make it difficult for quantification of phytohormones due to the high oil content. Lipids are difficult to avoid the co-extraction with the phytohormones in organic solvents during the preparing process, the detection sensitivity of the target analytes will be significantly reduced due to the existence of lipids [8]. The structural and chemical diversity of phytohormones make it challenge to simultaneous determination of multiple phytohormones. Therefore, a highly sensitive and reliable quantification method to determine multiple phytohormones in oilseeds rich in fatty acids is extremely demanding.

Nowadays, the ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is being widely acknowledged for analyzing the phytohormones [12]. Chemical-derivatization assisted LC-MS methods have been demonstrated to be powerful tools for sensitive detection of trace phytohormones with poor MS response in negative mode [13, 14]. Although derivatization reactions are tedious and time-consuming, various derivatization reactions have been used to enhance both the ionization efficiency and detection sensitivity of the phytohormones. Many of the reported derivatization reactions of acid phytohormones mostly occur in an organic medium [15, 16]. GAs, IAA, ABA, and JA contain carboxyl groups which could derivatize by reagents with a quaternary ammonium group such as N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), what's more, the EDC-derived phytohormones could be generated in an aqueous medium [17]. The implementation of *in situ* aqueous derivatization coupled with the sample pre-treatment technique for phytohormones might simplify the analytical methods. The combination of extraction and *in situ* derivatization is considered as a promising sample preparation method.

For the oilseeds, samples were often extracted and purified in multiple steps during the pre-treatment process to reduce the interference of lipid matrix [18]. In recent years, the matrix dispersion technology was developed for the treatment of fatty solid samples by mixing with clean-up sorbents [19]. The common dispersive sorbents used in fatty matrices include C18, primary secondary amine (PSA), and florisil [20]. Nowadays, magnetic dispersion solid-phase extraction (MD-SPE) is being applied to the plant samples for quantification of phytohormones [17, 21]. In brief, MD-SPE is more convenient than the conventional SPE due to the advantages of simplicity, time saving, and excellent adsorption efficiency [22]. Therefore, the highly selective and functional decorated adsorbent has attracted more attention to enrich the phytohormones. Ti_3C_2 MXene exerts a two-dimensional micro-crack structure allowing large specific surface area, high porosity, and good stability [23]. It has demonstrated that Ti_3C_2 exhibits a greater ability to adsorb a variety of environmental pollutants, including organic dyes, heavy metal ions, and gas molecules [24, 25]. β -Cyclodextrin (β -CD) is a type of oligosaccharide having a unique appearance of hydrophilic and inner cavity hydrophobic cup structure. The β -CD could selectively bind phytohormones with the special hydrophobic cavity [21]. The magnetic Ti_3C_2 composite conjoins the high adsorption capacity with the convenient magnetic separation. Supporting this statement, further study demonstrated that magnetic Ti_3C_2 can be modified by β -CD and is possible to improve the selectivity of phytohormones in

oilseeds samples. To the best of our knowledge, this is the first study reporting the use of $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ to selective enrichment of phytohormones. However, the application of functionalized Ti_3C_2 MXene for solid extraction adsorbent to the analysis of phytohormones needs to be further explored.

The present study aimed at developing a rapid, eco-friendly, and effective method based on the *in situ* derivatization and MD-SPE for the extraction of phytohormones in the oilseeds samples followed by LC-MS/MS determination. In this study, oilseeds samples and sorbent were ground by the matrix dispersion to decrease the fatty acids. The $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ was prepared as the sorbent material showing good extraction efficiency for phytohormones. The extraction, purification, and derivatization were integrated into one single step by adding an EDC derivatizing agent and performed in a micro-centrifuge tube without any sample transfer. The main parameters involved were optimized. Finally, the proposed method was successfully applied to different oilseeds.

Results And Discussion

Characterization of $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ composite

Fig. 1a illustrates the X-ray diffraction (XRD) patterns of the $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2$ and $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$. The characteristic diffraction peaks (002) of the material were fitted well with Ti_3C_2 , which was consistent with the report [26]. The characteristic diffraction peaks of Fe_3O_4 matched well with the standard XRD data of magnetite[22]. After $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2$ was grafted with $\beta\text{-CD}$, the pattern was unchanged in its peak positions, suggesting that the crystal structure of Fe_3O_4 nanoparticles was not affected by the grafted $\beta\text{-CD}$. The FT-IR of the composite material detected the relatively strong peaks of 3436 cm^{-1} , 1030 cm^{-1} , and 584 cm^{-1} (as shown in Fig. 1b), which should be from the stretching vibration of O-H, C-O of $\beta\text{-CD}$, and Fe-O of Fe_3O_4 , respectively [21, 22]. Fig. 1c illustrates the measurement of the field-dependent magnetization by a vibrating sample magnetometer at room temperature. The significant hysteresis loops in the S-shaped curves indicate the ferromagnetic behaviors of the materials. The saturation magnetization was calculated to be 66.0 emu/g .

The microstructures of the Ti_3C_2 and $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ were characterized by the scanning electron microscopy (SEM) and the transmission electron microscopy (TEM). Fig. 2a illustrates the typical Ti_3C_2 MXene with layered structure, whereas Fig. 2b displays the distribution of nanoparticles on the surface of multi-layered Ti_3C_2 MXene. As depicted in Fig. 2c, 2d, the transmission electron microscopy (TEM) image of the hybrid reveals the incorporation of the Fe_3O_4 nanoparticles between the space of multi-layered Ti_3C_2 . Elemental mapping can be found that Fe_3O_4 nanoparticles are homogeneously distributed on the Ti_3C_2 from element mapping of Fe and Ti (Fig. 2e).

Optimization of parameters in the simultaneous derivatization and MD-SPE

Selection of the clean-up sorbents

Lipid components are the most common coexisting matrix in the extraction phase of oilseeds. So the effects of different sorbents (C18, PSA, kieselguhr, alumina, and florisil) on extraction efficiency were investigated. As depicted in Fig. 3a, the effects of the florisil sorbent on extraction efficiency were significantly higher on the phytohormones compared to other clean-up sorbents. Florisil as matrix sorbent may be attributed to purify the oilseeds samples, also has advantageous effects on decrease the retention of targets. Thus, florisil sorbent was employed for the following experiments.

Selection of extraction solvent types and volumes

The selection of an appropriate extraction solvent can achieve higher extraction efficiency of phytohormones. In this study, four organic solvents (acetonitrile, methanol, 80% acetonitrile, and 80% methanol) were evaluated. As depicted in Fig. S1, methanol enhances the extraction efficiencies for multiple phytohormones. Hence, methanol was preferred as an appropriate extraction organic solvent. Further, the influences of the extraction solutions' volume on the phytohormones were investigated. As shown in Fig. S2, the extraction solution increased from $200\text{ }\mu\text{L}$ to $400\text{ }\mu\text{L}$ exhibiting a higher distribution ratio of the analytes in the organic phase, thereby decreasing the extraction efficiencies. Therefore, $200\text{ }\mu\text{L}$ of methanol was considered as the extraction solution.

Optimization of the amount of magnetic solid-phase extraction sorbents

The EDC-derived phytohormones possess a positively charged quaternary ammonium group that may attract the hydroxyl groups or oxygen groups existing on the surface of Ti_3C_2 [27]. At the same time, the $\beta\text{-CD}$ could selectively bind the phytohormones by molecular piston [28]. The effect of the amount of magnetic solid-phase extraction sorbents were investigated by adding different amounts $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$. As shown in Fig 3b, the peak area of the phytohormonal derivatives increased with the change of $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ from 1 mg to 5 mg . The increase in the adsorption capacity could attribute to the availability of a greater number of adsorption sites. Only a slight increase in the adsorption was observed when the sorbent amount exceeded 5 mg . Thus, 5 mg $\text{Fe}_3\text{O}_4/\text{Ti}_3\text{C}_2/\beta\text{-CD}$ nan-composite was used in the following experiments.

Optimization of simultaneous derivatization and magnetic solid-phase extraction time

The extraction and *in situ* derivatization processes were the pivotal steps in this method. Therefore, the effect of extraction and *in situ* derivatization time were investigated by varying from 30 to 120 min . As shown in Fig 3c, the signals of derivatives increased with the extension of reaction time until 90 min , and then insignificant changes were observed. Therefore, 90 min was chosen to mediate the extraction and *in situ* derivatization.

Optimization of desorption solution volume and desorption time

It should be noted that water is a strong desorption solvent in the hydrophilic retention systems [29]. Therefore, water was used as the desorption solution for the phytohormonal derivatives. The volume of the desorption water solution was further optimized. As shown in Fig S3, 50 μL of desorption solution was enough for the desorption of the derivatives from the sorbent. The dilution effect of the desorption was observed when the volume of the desorption solution increased from 50 μL to 100 μL . Hence, 50 μL water was used as the desorption solution. The optimization of desorption time ranged from 10 to 30 min was performed, which indicated that 20 min was enough for efficient desorption (Fig. 3d). The mass transfer between the sorbent and acidic phytohormonal derivatives may be completed within 20 min. Hence, the desorption time of 20 min was selected.

Method validation

As depicted in Table 1, the calibration curves of all the analytes exhibited good linear determination coefficients R^2 (≥ 0.9928). The obtained calibration curves demonstrate the excellent linearity for the range studied in this work. The LOD and LOQ values were within the range of 0.89-13.62 pg/mL and 2.99-45.39 pg/mL for all target analytes, respectively. In Table 1, the recoveries were ranged from 80.4% to 117.7% for all target phytohormones. The RSD values of the intra-day precisions and inter-day precisions were in the range of 1.1-13.7 % and 0.1-11.6 %, respectively. These results indicate the acceptable repeatability and reproducibility of the proposed method. A matrix-effect is considered to be a suppression or enhancement of the analyte response due to the presence of co-eluting matrix constituents during the chromatographic run [30]. Signal suppression or enhancement may exert negative or positive ME values, respectively. A ME < -20 % indicates high ion suppression whereas an ME > 20 % indicates high signal enhancement effect and -20 % $< \text{ME} < 20$ % indicates no matrix effects [31]. In this study, moderate signal suppressions (-16.63 % $< \text{ME} < 17.06$ %) were observed for all analytes. The absolute values of the ME were significantly low due to the derivatization or the matrix clean-up of florisil adsorbent. For example, the EDC derivatization could increase the target molecules' sensitivity avoiding matrix interference [32].

Applying of the proposed method for the analysis of different oilseed samples

The validated analytical method was employed to determine the content of phytohormones in different oilseeds plants (bean, peanut, and sesame). The total fatty acid contents of different oilseeds were shown in Fig S5. The concentrations of 12 phytohormones are listed in Table 3 ranging from ND (not detected) to 1289.5 ng/g FW. The total content of the phytohormones in bean was higher than in peanut and in sesame, especially for ABA. High content of ABA in bean is similar with the previously report [33]. The recovery experiments were carried out and the obtained results are depicted in Table 2. These recoveries and SD indicated that the present method is suitable for the determination of phytohormones in different oilseeds plants.

Comparison of the proposed method with other methods

In Table 3, the proposed method was compared with other solid-phase extraction methods for analyzing the phytohormones in the plant from previous reports. Graphene oxide [21], SiO_2 [34], and TiO_2 [17] were reported as the adsorbents for phytohormones during the solid-phase extraction. The MD-SPE is more convenient than the conventional SPE cartridges due to less consumption time and organic solvent [35]. These reported methods limited the plant sample consumption between 100 mg to 3.0 g FW for multi-phytohormonal profiles, but more sample requirements could make it difficult to detect the minute plant organs [21, 36, 37]. In contrast, this method provides quantification of most major plant hormones from a single rapeseed (4-6 mg). Compared with other solid-phase extraction coupled with UPLC-MS/MS methods, the LODs of this method were lower than the LODs of previously reported about for target phytohormones. $\text{Fe}_3\text{O}_4@ \text{Ti}_3\text{C}_2@ \beta\text{-CD}$ nano-composite material and EDC derivatization contributed to the increased detection sensitivity of the methods. This simple procedure deduced the losses from transfers and may ensure extractive and reliable recoveries (80.4-117.7 %). Hence, the proposed method has several advantages over the other reported techniques, being simple, effective, and eco-friendly.

Conclusion

In this study, the $\text{Fe}_3\text{O}_4/\text{Ti}_3\text{C}_2/\beta\text{-CD}$ was successfully synthesized and developed as the magnetic solid-phase extraction adsorbents for the selective extraction of multiple gibberellins and several phytohormones. A new version of prepared method concluded that the magnetic solid-phase combined with *in situ* EDC derivatization is the best approach for studying the distribution of 12 phytohormones in rapeseed as it provides simplification, adequate selectivity, and sensitivity. This method was also successful for analyzing phytohormones and resolving the problems such as instability, trace amount, and matrix interference in different oilseeds samples.

Methods

Chemical and reagents

Gibberellin A_1 (GA_1), GA_3 , GA_4 , GA_5 , GA_7 , GA_8 , GA_9 , GA_{20} , GA_{51} , IAA, ABA, and JA were purchased from OlChemIm Ltd. (Olomouc, Czech Republic). $[^2\text{H}_2]\text{GA}_1$, $[^2\text{H}_2]\text{GA}_4$, $[^2\text{H}_2]\text{GA}_9$, $[^2\text{H}_6]\text{ABA}$, $[^2\text{H}_5]\text{JA}$, and $[^2\text{H}_2]\text{IAA}$ were purchased from OlChemIm Ltd. (Olomouc, Czech Republic) and used as internal standards (ISTDs). EDC ($>98.0\%$) was purchased from TCI Development Co., Ltd., (Shanghai, China). Methanol ($\geq 99.9\%$) and acetonitrile ($\geq 99.9\%$) were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). The matrix dispersion sorbents for experiments included kieselguhr, C18-bonded silica (C18), primary secondary amine (PSA), alumina-N, and florisil were purchased from CNW Technologies (Shanghai, China). Ti_3AlC_2 powder (> 98 wt% purity) was obtained from 11 Technology Co., Ltd, China. Ultrapure water (resistivity ≥ 18.25 M Ω /cm) obtained from WaterPro water system (ULUPURE, China) was used in all experiments. Individual stock standard solutions (1000 mg/L) of each compound were prepared using methanol and stored in the refrigerator at -20 $^\circ\text{C}$.

The mixed standard solutions were stored at 4 °C. EDC solutions were prepared by freshly dissolving EDC powder in the methanol. All the reagents were of analytical grades (least 98% purity).

Preparation of magnetic nano-composite Fe₃O₄@Ti₃C₂@β-CD

Fig.4 illustrates the method of preparation of Fe₃O₄@Ti₃C₂@β-CD hybrid. 2 g of the Ti₃AlC₂ powder was immersed in 20 mL of hydrofluoric acid (HF) solutions by stirring for 24 h at 60 °C for complete removal of the Al layers. Then, the solution was centrifuged and rinsed several times using deionized water to remove the HF and until the pH attained 5-6. The obtained Ti₃C₂ powder was then vacuum-dried at 80 °C for 24 h.

Fe₃O₄ nanoparticles were synthesized according to the hydrothermal method [38]. 100 mg of Ti₃C₂ powder and 50 mg of Fe₃O₄ nanoparticle were ultrasonically dispersed in 80 mL and 20 mL of deionized water for 30 min, respectively. Both were mixed by ultrasonification for 120 min and argon shield. Then the suspension was filtrated to obtain the Fe₃O₄@Ti₃C₂ hybrid and followed drying in a vacuum at 50 °C for 24 h.

Fe₃O₄@Ti₃C₂ composite loaded with β-CD, 100 mg of Fe₃O₄@Ti₃C₂ and 2.0 g of β-CD were dispersed into 60 mL deoxygenated water by ultrasonification for 20 min. Then, the reaction system was kept in an oil bath at 60 °C for 4 h and argon shield. Finally, the obtained Fe₃O₄@Ti₃C₂@β-CD was washed with deionized water and then vacuum-dried at 50 °C for 24 h.

Plant materials

Seeds of *Brassica napus* (rapeseed), *Sesamum indicum* (sesame), *Glycine max* (bean), and *Arachis hypogaea* (peanut) were obtained from Hunan Branch of National Center of Oilseed Crops Improvement (Changsha, China). The different seeds of oil crops were harvested, frozen in liquid nitrogen, and stored at -80 °C until further analyzed.

Sample preparation and *in situ* derivatization

Single fresh rapeseed seed was mixed with 2 mg clean-up sorbents and beads into a 2.0 mL microcentrifuge tube. The mixture was immediately ground with one cold ZrO₂ mill ball by ball milling (TissueLyser II, QIAGEN, Germany) at 20 Hz for 4 min. Then, the internal standards (IS) were added into the tube. The ground sample was extracted with 200 μL of cold methanol and vortexed at 4 °C for 30 S, then left to stand at 4 °C for 12 h. The supernatant was collected by centrifugation at 10000 ×g for 5 min and transferred to a 1.5 mL microcentrifuge tube. The supernatant was added with 5 mg magnetic nanoparticles and 50 μL of derivatizing agent (20 mM EDC), then incubated at 40 °C for 90 min. Both the extraction and derivatization were completed at one step. The nanoparticles were collected from the suspension by magnetic separation and re-dispersed by 50 μL of 5 % menthol. After desorption for 20 min, the eluent was collected and determined by UHPLC-ESI-MS/MS. The general process of sample pre-treatment is illustrated in Fig.4.

Instruments and analytical conditions

The UPLC-MS/MS was equipped with an Agilent 1290 series (Agilent Technologies, USA) and Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, USA). The analytes were separated on a Waters ACQUITY UPLC HSS T3 column (100 mm × 2.1 μm). The optimized separation conditions were as follows: the column oven temperature was kept at 40 °C, and the sample injection volume was 10 μL, the flow rate of the mobile phase was 0.3 mL/min. The elution gradient program of the positive ion mode was performed, as depicted in Table S1.

The multiple reaction monitoring (MRM) was employed for the quantitative analysis of the targeted compounds. Nitrogen gas was used as the drying and collision gas. The ionization source conditions were as follows: the flow rate of the nebulizer gas was 8 L/min, the source temperature of the mass spectrometer was 300 °C, the nebulizer pressure was 50 psi, and the capillary voltage was 3500 V. The details of the EDC-derived phytohormones and their optimized MRM parameters are listed in Table 4. The MRM chromatograms of the target EDC-derived phytohormones were shown in Fig. 5. The MRM chromatograms with subsection of target phytohormone derivates in a single rapeseed were shown in Fig. 6.

Method validation

To validate the developed method, the linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and matrix effect (ME) were investigated. Calibration samples at 6 concentrations (0.01, 0.05, 0.10, 0.50, 1.0, 10 ng/mL of GAs derivates, 0.10, 0.50, 1.0, 5.0, 10, 100 ng/mL of IAA, ABA, JA derivates) with a fixed concentration of IS ([²H₂] GA₁ 0.1 ng/g, [²H₂] GA₄ 0.1 ng/g, [²H₂] GA₉ 0.1 ng/g, [²H₂] IAA 0.2 ng/g, [²H₆] ABA 1 ng/g, and [²H₆] JA 0.1 ng/g for each). The LOD values were calculated as 3 times of the standard deviation of 11 replicates for rapeseed samples spiked at the 0.01 ng/mL level for GAs, 1 ng/mL level for IAA, ABA, and JA, respectively. LOQ values were calculated as 10 times of the standard deviation of 11 replicates for rapeseed samples spiked at the same level as used in calculating the LOD values.

Accuracy was evaluated by the recovery of each target analyte at low, medium, and high levels, respectively. The precision was investigated by the intra-day precision (repeatability) and inter-day precision (reproducibility). The intra-day precision was evaluated by analyzing 7 replicates on the same day while the inter-day precision was carried out for 3 consecutive days (7 replicates per day). During the trace analyses with a complex matrix, the ME usually occurs and its percentage can be calculated as per the equation below:

$$ME\% = (A_{\text{extract}}/A_{\text{solvent}} - 1) \times 100$$

where, A_{extract} stands for the slope of matrix-match calibration curves, A_{solvent} stands for the slope of solvent calibration curves.

Declarations

Authors' contributions

ZL designed the work; XW performed the experiments; ZL, XW and XL processed the experiment data; ZL wrote the manuscript; LX and RW revised and supervised the manuscript. ZL and MX contributed equally to this work. All authors read and approved the final manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The scripts and datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Tables

Table 1: Results of the method performance and validation study

Analytes	Linear range (ng/mL)	R ²	LOD ^a (pg/mL)	LOQ ^b (pg/mL)	Intra-day precision (%) ^c			Inter-day precision (%) ^c			Recoveries(%,n=3)			ME ^d %
					Low	Medium	High	Low	Medium	High	Low	Medium	High	
GA ₁	0.01-10	0.9989	0.89	2.99	7.1	8.2	1.5	11.6	0.7	5.4	104.0	90.4	108.5	-16.63
GA ₃	0.01-10	0.9994	3.91	13.05	8.0	9.8	2.0	8.7	4.7	5.7	105.0	101.2	97.7	-5.21
GA ₄	0.01-10	0.9993	10.52	35.08	7.1	3.5	10.1	4.0	3.9	4.8	115.1	117.7	99.8	17.06
GA ₅	0.01-10	0.9987	5.70	19.00	2.0	1.5	6.7	11.2	4.3	3.4	85.3	103.6	104.1	15.33
GA ₇	0.01-10	0.9996	12.62	42.09	10.4	12.3	6.2	4.9	9.2	6.7	95.5	94.3	88.4	-15.80
GA ₈	0.01-10	0.9928	5.37	17.90	13.7	3.0	3.4	2.3	7.3	3.3	112.1	99.4	104.3	4.17
GA ₉	0.01-10	0.9993	1.89	6.30	1.2	2.9	11.7	5.0	7.8	4.6	111.8	99.5	91.5	1.18
GA ₂₀	0.01-10	0.9977	7.80	26.02	7.3	5.7	10.1	6.4	5.1	0.1	94.7	110.8	109.7	0.72
GA ₅₁	0.01-10	0.9989	0.65	2.18	3.4	1.1	11.4	6.4	7.9	2.9	80.4	98.5	87.2	-2.72
ABA	0.1-100	0.9991	7.59	25.31	9.5	15.1	2.5	9.7	7.4	9.1	108.6	89.3	92.1	0.29
JA	0.1-100	0.9991	2.31	7.70	8.9	9.7	5.2	10.8	7.8	1.7	92.2	102.7	100.9	-4.83
IAA	0.1-100	0.9996	13.62	45.39	13.5	5.4	5.0	2.2	3.7	11.0	91.6	107.2	102.5	2.14

^a LOD: limit of detection;

^b LOQ: limit of quantification;

^c Phytohormones standards were spiked in rapeseed at three different concentrations (2, 10, 20 ng/g FW for GA_s, 10, 20, 100 ng/g FW for ABA, JA, IAA);

^d ME: matrix effect.

Table 2: Results (means ± standard deviation) obtained for the phytohormones detection and recovery in different oilseeds

Analytes	Bean		Peanut		Sesame	
	Determined (ng/g)	Recovery (%) ^a	Determined (ng/g)	Recovery (%)	Determined (ng/g)	Recovery (%)
GA ₁	4.5± 0.4	103.2 ±5.2	10.2±0.6	111.1 ±5.0	ND	102.1±9.7
GA ₃	ND	100.4 ±10.6	28.2 ±2.0	102.1 ±5.2	ND	101.8 ±6.1
GA ₄	9.3 ±0.8	114.3 ±1.6	7.3 ±0.3	110.8 ±0.8	5.3±0.7	110.9 ±5.9
GA ₅	ND	104.9 ±6.8	17.6 ±1.2	101.2 ±8.3	ND	109.0 ±0.6
GA ₇	ND	94.3 ±1.1	ND	92.5 ±4.8	ND	109.1±10.3
GA ₈	ND	97.5 ±6.3	28.2±1.6	109.5 ±6.1	ND	120.2±2.0
GA ₉	ND	80.4±9.2	4.6 ±0.2	92.0 ±2.4	ND	81.3±3.4
GA ₂₀	16.9 ±2.3	96.1 ±1.5	28.6 ±0.9	112.8 ±6.5	ND	118.4±2.2
GA ₅₁	ND	89.5 ±0.3	ND	110.0 ±0.7	ND	84.8±2.3
ABA	1289.5±6.1	108.0 ±7.7	342.9±11.7	86.3 ±3.5	ND	99.7 ±8.7
JA	ND	116.4 ±0.7	ND	114.8 ±3.0	ND	107.3 ±5.1
IAA	10.9 ±0.6	105.4 ±7.4	ND	106.9 ±2.2	20.1 ±1.3	90.3 ±4.4

ND: not detected;

^a : the recovery was calculated at the spiked of 20 ng/g for GAs, 100 ng/g for ABA, JA, and IAA.

Table 3: Comparison of the solid phase extraction method with other methods of the phytohormones

Sample	Analytes	Solid phase extraction adsorbent	Derivatization	Method	LOD	Recovery (%)	Reference
1.0 g oilseed rape leaves	IAA, ABA, SA, JA, GAs, CKs, and 6-BA	Oasis MCX cartridges	-	HPLC-MS/MS	1.3 -21.0 pg/mL	75.1-113%	[38]
100 mg flower	IAA, ABA, JA, 4 GAs, 3 BRs, and 6 CKs	Fe ₃ O ₄ @SiO ₂ @Poly(DMAPBA-co-EGDMA)	-	UHPLC-MS/MS	1.9-59.6 pg/mL	85.0-116.2%	[36]
300 mg Arabidopsis	IAA, ABA	SiO ₂ @GO	-	HPLC	30-50 pg/mL	91.8-118.4%	[34]
3.0 g tomato	NAA, 2-NOA	Fe ₃ O ₄ /rGO@β-CD	-	HPLC-MS/MS	0.67 ng/g	91.5-95.9%	[21]
100 mg rice seeds	8 CKs, IAA, ABA, JA, and 10 GAs	Fe ₃ O ₄ @TiO ₂	-	UHPLC-MS/MS	0.01-1.99 fmol	86.8-118.1%	[37]
100 mg rice leaves	GA _s , JA, IAA, ABA	TiO ₂ /magnetic hollow mesoporous silica sphere	BTA	UHPLC-MS/MS	1.03-91.21 pg/mL	71.6-112.8%	[17]
5 mg oilseeds	9 GAs, IAA, JA, ABA	Fe ₃ O ₄ /Ti ₃ C ₂ /β-CD	<i>In situ</i> EDC derivatization	UHPLC-MS/MS	0.89-13.62 pg/mL	80.4-117.7 %	This method

NAA: 1-naphthalene acetic acid; 2-NOA: 2-naphthoxyacetic acid; GO: graphene oxide, BTA: 13-bromoactonyltrimethylammonium bromide, EDC: N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide.

Table 4: Details of the EDC-derived acidic phytohormones and their MRM parameters under positive ionization mode

Analytes	Internal standards	RT [min]	Parent ion (m/z)	Product ion (m/z)	Collision energy (eV)	Fragmentor (V)
GA ₁ -EDC	[² H ₂]GA ₁	7.6	504.2	433.3/388.8	25/30	130
GA ₃ -EDC	[² H ₂]GA ₁	7.4	502.2	431.3/386.2	20/28	135
GA ₄ -EDC	[² H ₂]GA ₄	17.5	488.3	417.4/372.2	28/35	135
GA ₅ -EDC	[² H ₂]GA ₄	12.1	486.3	415.0/370.1	20/27	135
GA ₇ -EDC	[² H ₂]GA ₄	17.2	486.3	415.1/370.1	20/30	135
GA ₈ -EDC	[² H ₂]GA ₁	3.8	520.3	449.4/404.2	22/32	130
GA ₉ -EDC	[² H ₂]GA ₉	20.0	472.3	401.2/356.2	20/30	130
GA ₂₀ -EDC	[² H ₂]GA ₄	12.5	488.4	417.5/372.2	22/33	135
GA ₅₁ -EDC	[² H ₂]GA ₄	14.5	488.3	417.1/372.2	25/35	135
ABA-EDC	[² H ₆]ABA	11.2	420.3	349.3/304.3	15/25	135
JA-EDC	[² H ₅]JA	16.0	366.3	250.2/329.2	20/12	120
IAA-EDC	[² H ₅]IAA	10.9	331.2	215.2/260.2	18/10	120
[² H ₂]GA ₁ -EDC	-	7.6	506.4	435.4/390.3	20/34	135
[² H ₂]GA ₄ -EDC	-	17.5	490.4	419.4/374.2	25/28	135
[² H ₂]GA ₉ -EDC	-	20.4	474.4	403.4/358.2	20/33	135
[² H ₆]ABA-EDC	-	11.1	426.3	355.1/310.4	17/23	135
[² H ₅]JA-EDC	-	16.0	371.4	300.3/255.2	12/20	120
[² H ₂]IAA-EDC	-	10.8	336.3	220.3/265.3	18/10	100

Figures

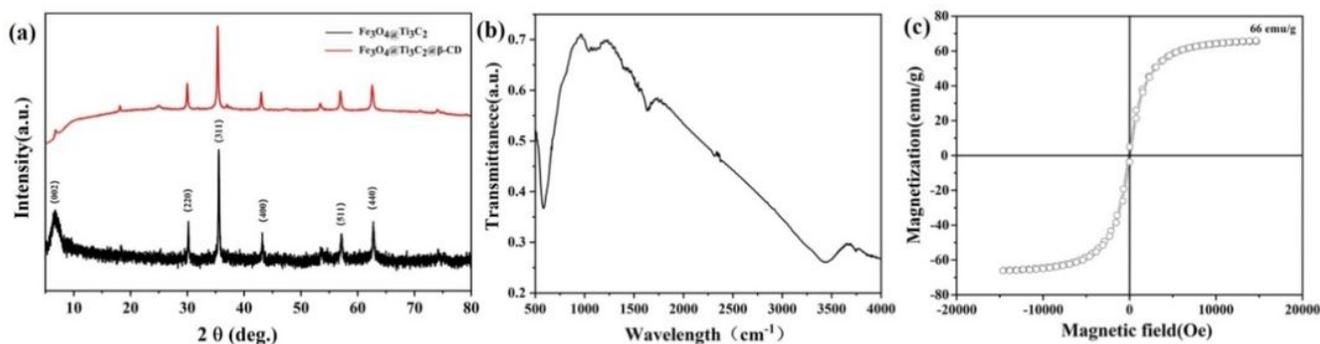


Figure 1

XRD spectrum (a), FT-TR pattern (b), magnetization hysteresis loop (c) of Fe₃O₄@Ti₃C₂@β-CD

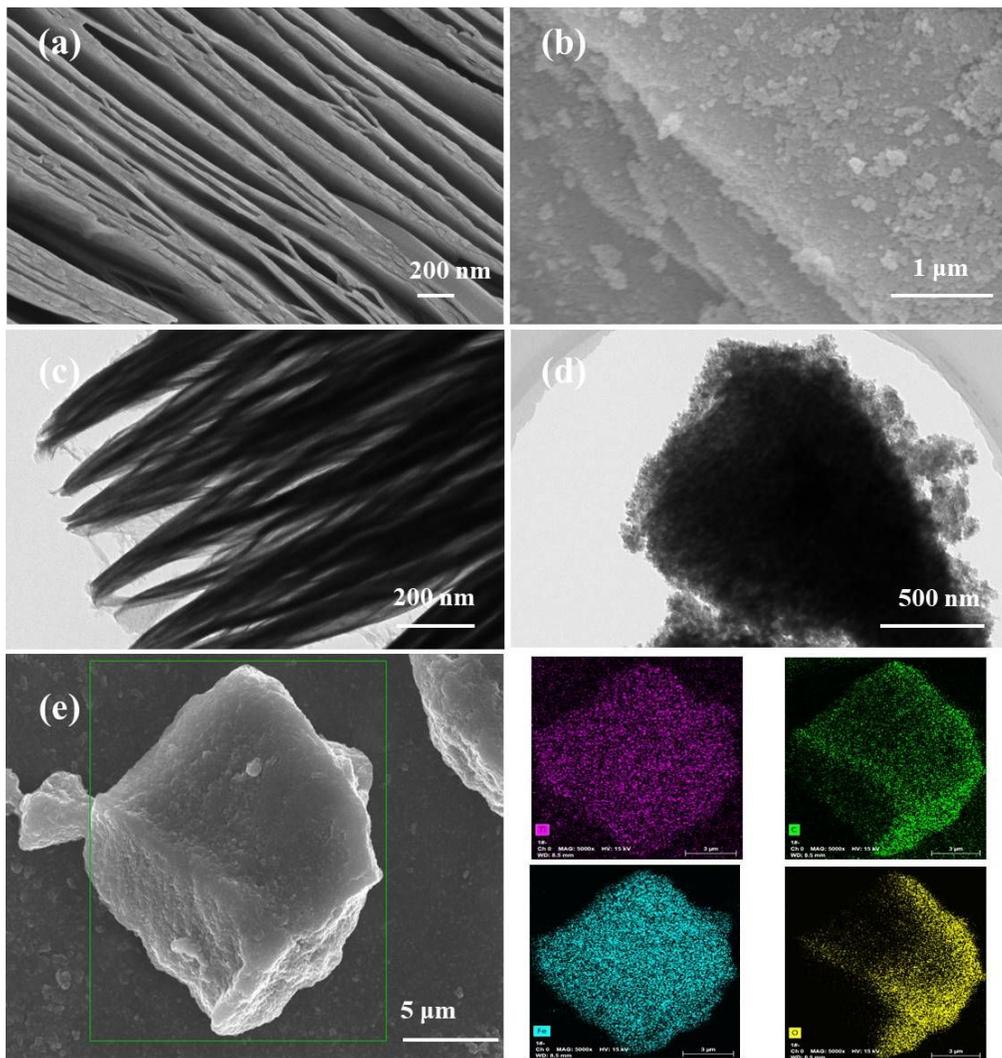


Figure 2

SEM image of Ti₃C₂ (a), SEM image of Fe₃O₄@Ti₃C₂@β-CD (b), TEM image of Ti₃C₂(c), TEM image of Fe₃O₄@Ti₃C₂@β-CD (d), elemental mapping and chemical composition of Fe₃O₄@Ti₃C₂@β-CD (e)

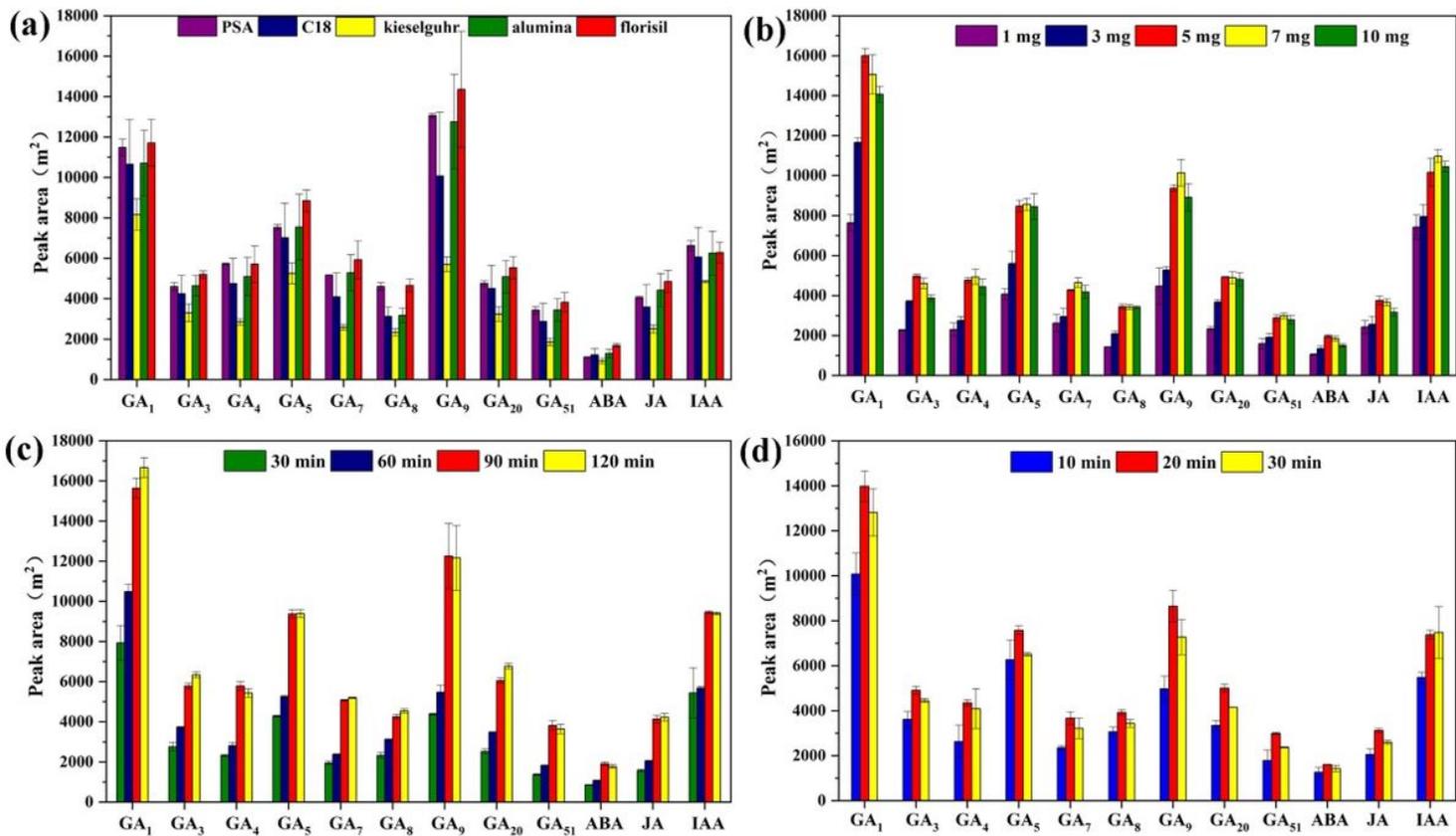


Figure 3
 Effects of different cleanup sorbents (a), effects of the amount of magnetic solid-phase extraction sorbents (b), effects of the simultaneous derivatization and magnetic solid phase extraction time (c), effects of the desorption time (d). 5 mg rapeseed spiked with 10 ng/g of each analyte

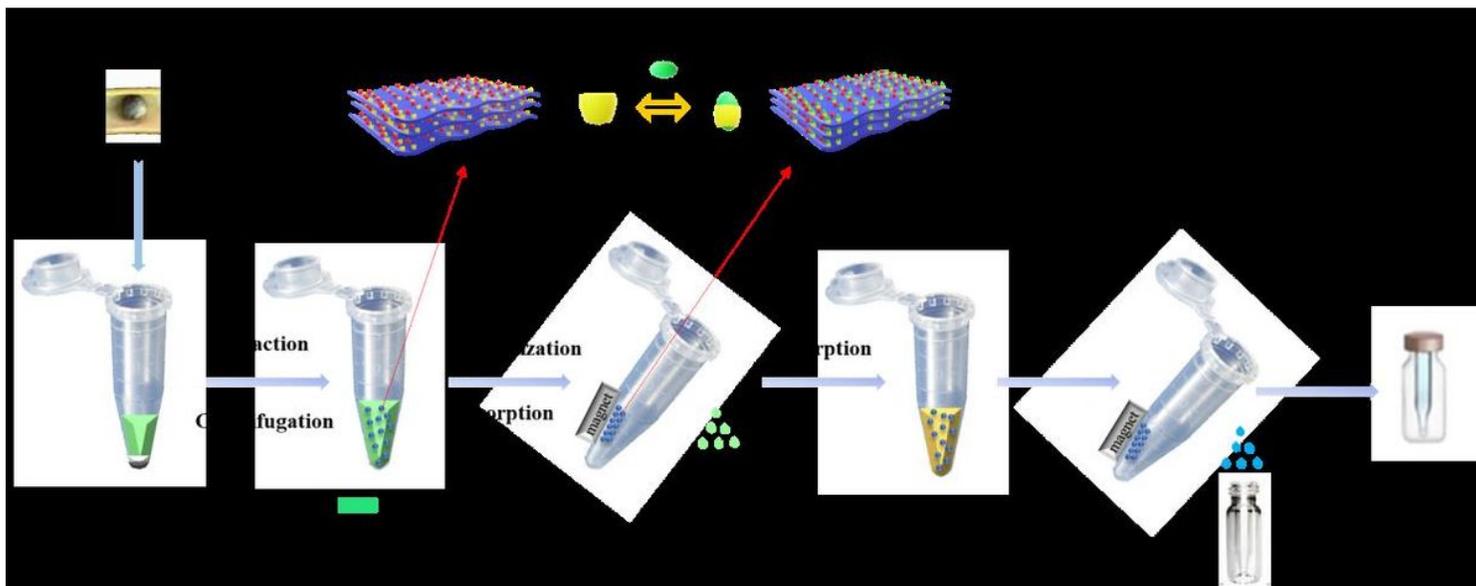
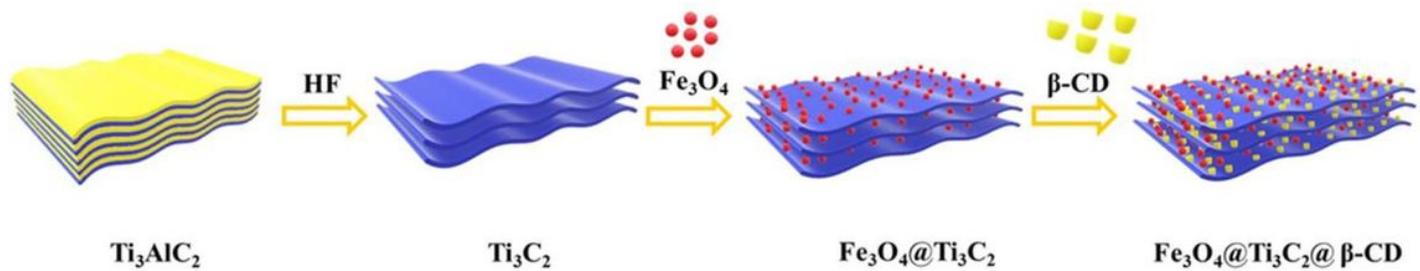


Figure 4

Schematic of the synthetic route for $\text{Fe}_3\text{O}_4@\text{Ti}_3\text{C}_2@\beta\text{-CD}$ and the magnetic solid phase extraction and in situ derivatization procedure

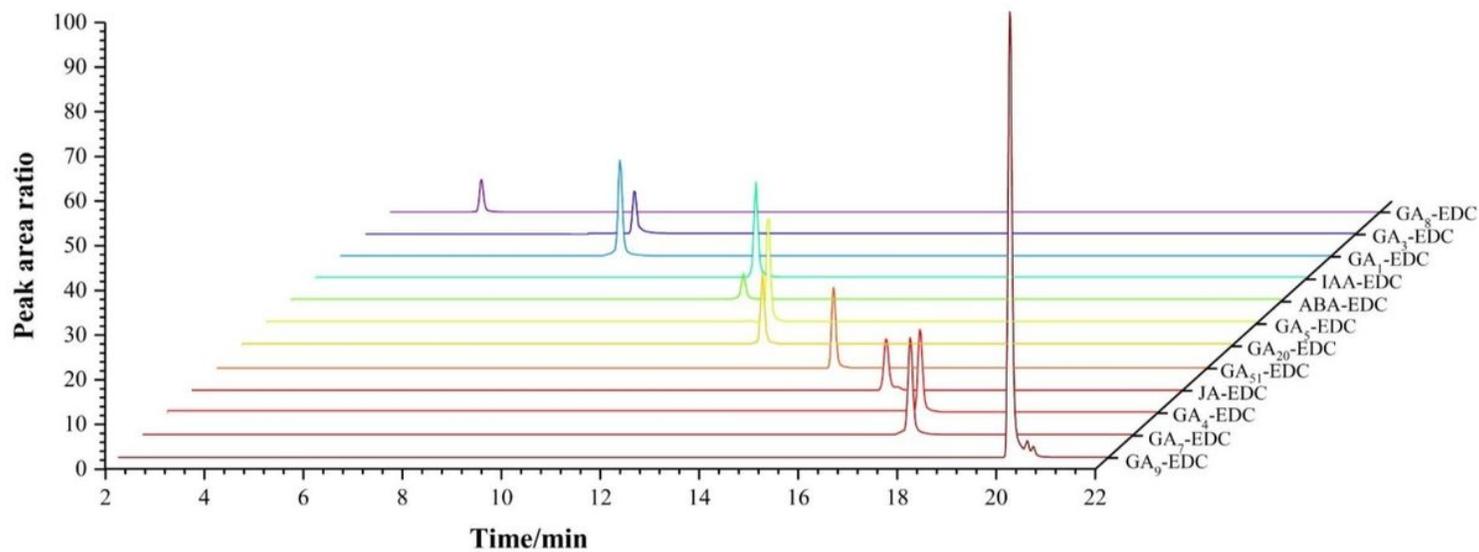


Figure 5

The MRM chromatograms of phytohormone standards (100 ng/mL)

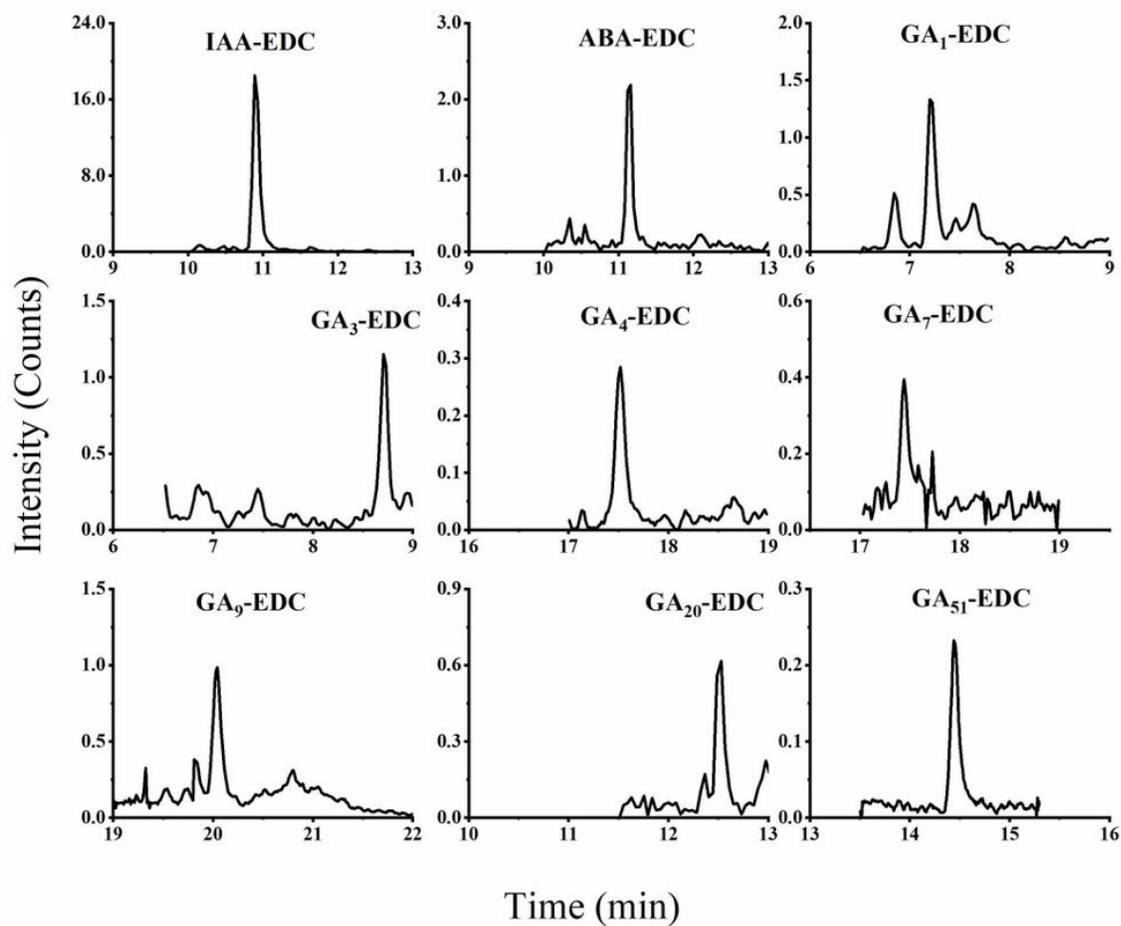


Figure 6

The MRM chromatograms of target phytohormones in a single rapeseed

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