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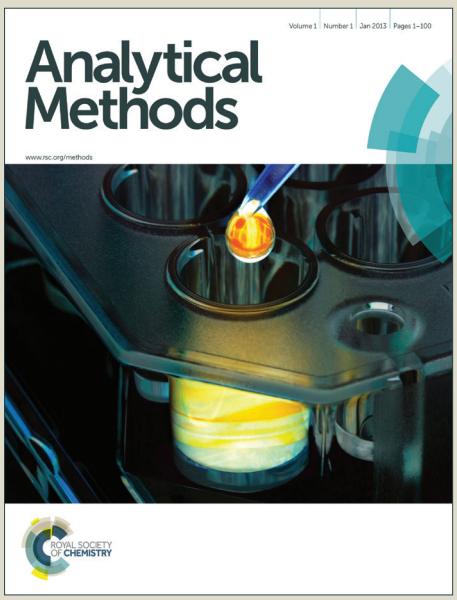

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Paper

Simultaneous determination of 10 plant growth promoters in fruits and vegetables with a modified QuEChERS based liquid chromatography tandem mass spectrometry method

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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A method was proposed for the multi-residue analysis of 10 widely used plant growth promoters (PGPs) based on liquid chromatography-tandem mass spectrometry (LC-MS/MS). The original QuEChERS (quick, easy, cheap, effective, rugged and safe) method was modified to reach the best recoveries, and the fast polarity switching mode of mass spectrometry was used to enlarge the scope of detection and improve the response of target compounds. The calibration curves showed good linearity in the range of 5.0 to 200.0 μg/L with correlation coefficients in excess of 0.990. No significant matrix effects were observed. Recoveries of 81-113% were obtained for all 10 PGPs with RSD values lower than 15% at three concentration levels in tomato, cucumber, watermelon and apple. Limits of detection and limits of quantification were ranged from 0.1 to 1.5 μg/kg and 0.3 to 5.0 μg/kg, respectively. The developed method offered fast and safe alternative to typical multi-PGPs analysis methods for fruit and vegetable samples with high recoveries and small susceptibility to matrix effects.

1 Introduction

Plant growth promoters (PGPs), a class of plant growth regulators, are well-known highly functional. Even at trace quantities, they can trigger a variety of basic physiological processes involved in cell division, cell enlargement, pattern formation, tropic growth, flowering, fruiting and seed formation.[1] In the past decades, some synthetic PGPs have appeared to be extensively used in edible plants in many countries, such as Australia, Japan, China and India[2]. However, residues remained at the harvest stage would produce hazardous effects on humans and/or environment[3], which has been an increased concern in recent years. To protect people against contamination and potential negative health effects, many countries and international organizations have regulated the maximum residue limits (MRLs) for some PGPs in appointed edible foods. As a consequence, food commodities demand a rigorous control to assure the non-violation of the MRLs, and there is a crucial need for multiresidue extraction and detection methods.

To analyze PGPs, series of methods have been proposed based on various techniques, i.e. high performance liquid chromatography (HPLC)[4, 5], gas chromatography (GC)[6], micellar-stabilized room temperature phosphorescence[7], first derivation synchronous fluorescence spectroscopy[8], gas chromatography–mass spectrometry (GC-MS)[9, 10] and liquid chromatography-tandem mass spectrometry (LC-MS/MS)[11-16]. Among them, LC-MS/MS allows for a quick and efficient determination of many compounds that cannot be determined easily and/or require laborious and slow techniques with conventional GC and LC methods. In addition, the stability, sensitivity and selectivity can be improved, the linear range can be extended, and false positive results can be discarded easily using multiple-reaction monitoring (MRM) mode.

However, sample preparation is still a major challenge to improve the accuracy and reliability of the results due to complex matrices in samples with diverse chemical properties and the strong polarities of most PGPs. To overcome these problems, many sample-pretreatment methods have been developed, including solid-phase extraction[17], solid-liquid extraction[18, 19], liquid-liquid extraction[20], liquid-liquid microextraction[21], solid-phase microextraction[22], matrix solid-phase dispersion[23], dispersive solid-phase extraction[24], stir bar sorptive extraction[25] and gel permeation chromatography[26]. However, most of them failed in multiresidue determination and yielded more consumption of toxic organic reagent and time, higher detection limits and poorer repeatability. In view of these points, a QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was introduced and raised much attention due to its several advantages of acceptable recoveries for acidic, neutral and basic pesticides, short time and low organic solvent consumption[27-31]. Recently, this method has been applied into various fields such as pesticides[32, 33], veterinary drugs[34] and mycotoxins[35] in different matrixes. Furthermore, it has been successfully applied into

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the determination of multi-PGPs in real samples[2, 36, 37]. To improve the recoveries of target compounds, various modified versions of the QuEChERS methods have been developed by modifying sorbents, such as primary secondary amine (PSA), C_{18}, calixarenes[38], graphitized carbon black or florisil[39, 40], *etc.* or using assisted extraction techniques, e.g. microwave[41] and ultrasound[42].

In this work, a modified QuEChERS method is developed for the simultaneous determination of ten commonly used PGPs in fruits and vegetables, including 6-benzyladenine (6-BA), indole-3-butyric acid, 1-naphthylacetic acid, 1-naphthylacetic methyl ester, 2-(1-Naphthyl)acetamide, forchlorfenuron, ethychlozate, 4-chlorophenoxy-acetic acid, 2,4-dichlorphenoxyacetic acid (2,4-D) and gibberellic acid (GA3). The extractant of acetonitrile (MeCN) is acidized, the step of ultrasound extraction is added and the amount of PSA is reduced. Higher recoveries and better precision demonstrate that the modified QuEChERS approach is very feasible. We expect that the method will be extensively used as a fast and effective multiresidue methodology for the detection of involved PGPs in fruits and vegetables in a near future.

2 Material and methods

2.1 Reagents and chemicals

Certified standards 6-BA, indole-3-butyric acid, 1-naphthylacetic acid, 1-naphthylacetic methyl ester, 2-(1-Naphthyl)acetamide, forchlorfenuron, ethychlozate, 4-chlorophenoxy-acetic acid, 2,4-D and GA3 were purchased either from Dr. Ehrenstorfer or from Sigma-Aldrich with the highest available purity. HPLC-grade MeCN and methanol were obtained from Fisher Scientific (Pittsburgh, PA). Acetic acid (content>99.7%) and ammonium acetate (content >99.5%) of HPLC grade were purchased from Sigma-Aldrich. Anhydrous magnesium sulfate and sodium acetate of analytical grade were obtained from the Chemical Reagent Company (Beijing, China). PSA was obtained from Agela Technologies (Beijing, China). Highly purified water (Milli-Q, Millipore, Bedford, MA) was used throughout the preparation of the mobile phase.

2.2 Standard solution preparation

Single standard stock solutions were prepared by dissolving 10.0 mg of the standards in 10 mL MeCN, respectively. An intermediate stock standard mixture of 10 mg/L was prepared by mixing individual stock solution and diluting in MeCN. Standard mixtures were prepared by diluting the individual stock solution in a diluted MeCN, which contains a certain amount of 0.1 % acetic acid. According to this procedure, solutions with several different concentrations (5.0, 10.0, 20.0, 50.0 and 200.0 μg/L) were obtained. The single and multicompound standards were stored at -18°C in the dark.

2.3 Sample preparation

Fruit and vegetable samples were prepared as follows: After being processed with a homogenizer, 10 g homogenate was weighed into a 50 mL plastic centrifuge tube. With the addition of 10 mL of 0.1% acetic acid in MeCN, the tube was vigorously shaken using a vortex mixer and then treated by an ultrasonicator for 30 min. Afterward, 4.0 g of $MgSO_4$ and 1.0 g NaAc were added. The mixture was shaken quickly to prevent formation of $MgSO_4$ conglomerates and then centrifuged for 5 min at 5000 rpm. After that, a 5 mL aliquot was transferred into a clean plastic centrifuge tube containing 50 mg PSA and 150 mg $MgSO_4$. And the mixture was shaken for 1 min, centrifuged for 5 min at 5000 rpm. Finally, the solution was filtered through a 0.22 μm membrane prior to the HPLC–MS/MS analysis.

2.4 Chromatographic and mass spectrometric conditions

Chromatographic analysis was conducted using a Thermo Scientific Surveyor HPLC system equipped with a vacuum degasser, a quaternary pump, a column oven, an auto sampler and a diode array detector. Chromatographic separation was achieved using a thermo hypersil GOLD aQ column (2.1 mm×150 mm, with particle size of 5.0 μm) at a flow rate of 200 μL/min. The binary solvent system consisted of water (A) and methanol (B) with a linear gradient. The linear mobile phase gradient started at 50% B (0-4 min), increased to 90% B (4-5 min), kept at 90% B (5-9 min), then ramped back to 50% B (9-10 min) and kept this ratio for 6 min to ensure all substrates drain out. The column temperature was maintained at 30°C. The injection volume was 5 μL and to avoid carry over, the autosampler was flushed with methanol between analytical runs. A divert valve was placed between the analytical column outlet and the mass spectrometer inlet, and the flow was diverted to waste during the first 1.0 min of the chromatographic run.

The HPLC system was interfaced to a triple quadrupole mass spectrometer with an ESI source (TSQ Quantum Access, Thermo Scientific). The analysis was determined in MRM mode using two mass transitions in addition to their relative abundances. Source parameters in positive mode were optimized as follows: spray voltage, 4.0 kV; sheath air pressure, 40 psi; auxiliary gas pressure, 10 psi; capillary temperature, 275°C. As for negative mode, spray voltage was 3.5 kV, and the other parameters were identified with positive mode. Data acquisition was performed under time-segmented conditions based on the chromatographic separation of the target compounds to maximize sensitivity of detection. Segment 1-4 min was detected in the negative-ion mode, and segment 4-16 min was detected in the positive-ion mode after a polarity switching.

2.5 Method validation

The following parameters, matrix effect, linear range, limit of detection (LOD), limit of quantification (LOQ), accuracy (%, recovery), and precision (%, RSD), were evaluated using HPLC-MS/MS.

2.5.1 Matrix effect

Blank abstracts (tomato, cucumber, apple and watermelon) were firstly analyzed to test the selectivity of the method. Matrix effect was evaluated by comparing the response of each pesticide obtained from a standard solution in solvent

and that from a spiked sample, and the corresponding slope in matrix/slope in solvent ratio was calculated.

2.5.2 Linearity study, LOD and LOQ determinations

The evaluation of the analytical curves' linearity was done based on injections of the standard solutions prepared in organic solvent (MeCN containing 0.1 % acetic acid) and also in blank extracts at the concentrations of 5.0, 10.0, 20.0, 50.0 and 200.0 μg/L, where the sequence was injected six times (n = 6). The corresponding range of pesticide concentrations in extract is from 5 to 200 μg/kg. Linear ranges, calibration curve equations and determination coefficients were calculated for each pesticide analyzed. LOD was calculated as three times of the signal-to-noise ratio, and LOQ was evaluated as ten times of the signal-to-noise ratio.

2.5.3 Accuracy and precision

The accuracy of the method was evaluated based on recoveries of pesticides. Three levels of mixture standard solutions were spiked into blank matirx at levels of 5, 20 and 100 μg/kg. After equilibrated 1 h to allow the spiked solution penetrated the matrix, the samples were treated according to above procedures, and then analyzed by LC-MS/MS. Recoveries were calculated from the ratio of the peak area of the analytes in sample extracts to the peak area of an equivalent amount of the standard solution. Intra-assay precision was evaluated by six-replicated analysis of blank samples fortified with target analytes at three concentration levels in one day, and inter-assay precision was derived from the analytical results of two replicates at three different concentrations in 10 consecutive days by different operators.

3. Results and discussion

3.1 Optimization of LC–MS/MS Conditions

Firstly, the mass parameters were tuned in positive or negative ionization mode for the analytes by infusing each standard solution of 10 mg/L into the mobile phase using a syringe pump, respectively. It was found that 1-naphthylacetic methyl ester and 2-(1-naphthyl)acetamide could be ionized only in positive mode and 4-chlorophenoxy-acetic acid, GA3, 2,4-D and 1-naphthylacetic acid only in negative mode. Meanwhile the other four target compounds, 6-BA, indole-3-butyric acid, forchlorfenuron and ethychlozate, could be ionized in both modes, but the responses of them were much higher in ESI positive mode than in negative mode. The results showed that the ten PGPs cannot be determined only with positive or negative mode. Luckily, the polarity switching strategy has been developed in modern LC–MS/MS systems, and showed great advantages to detect multiple analytes with different ionization polarities[24, 43, 44]. Here, it was also applied to simultaneously determine these ten PGPs with different ionization polarities to achieve the best sensitivity. The optimized LC-MA/MS condition was set as the negative mode at 1-4 min for 1-naphthylacetic acid, 4-chlorophenoxy-acetic acid, 2,4-D and GA3, and the positive mode at 4-16 min for indole-3-butyric acid, 6-benzyladenine, forchlorfenuron, ethychlozate, 1-naphthylacetic methyl ester and 2-(1-Naphthyl)acetamide.

According to European Union SANCO/12571/2013 guidelines[45], the ratio of the quantification/confirmation transitions in the sample and the previously injected standard should not differ by more than the percentage stipulated. Therefore, two most sensitive transitions in MRM mode were selected for each compound and listed in Table 1. The scan mode, MRM transitions, tube lens offset and collision energy (CE) were also summarized in Table 1.

The composition of the mobile phase can strongly influence the performance of the ionization process in the development of the LC-MS/MS methodology[12, 46]. Here, the effect of acetic acid and ammonium acetate, two most widely used modifiers, were also investigated. The results showed that both of them can improve the chromatographic resolution of analytes, but the MS signal response of some PGPs would be significantly decreased. As a comprise, methanol-water system, no modifier added, was selected. The resulted quantification MRM transitions of 10 PGPs were represented in Figure 1. .

3.2 Sample extraction

The original QuEChERS method consists of initial extraction with MeCN, followed by being partitioned after the addition of adequately mixed salts (anhydrous magnesium sulfate and sodium chloride) and subsequently being submitted to a DSPE clean-up step. For various analytes in different food matrices,

Table 1 MRM data acquisition parameters of HPLC-MS/MS for the 10 analytes

Compound	Scan mode	Quantification MRM 1	confirmation MRM2	Tube lens offset V	CE1 eV	CE2 eV
2,4-D	ESI-	219→161	219→125	90	28	17
ethychlozate	ESI+	239→165	239→138	94	17	34
2-(1-Naphthyl)acetamide	ESI+	186→141	186→115	116	17	36
4-chlorophenoxyacetic acid	ESI-	185→127	185→127	75	18	18
GA3	ESI-	345→239	345→143	122	40	18
1-naphthylacetic acid	ESI-	185→141	185→141	64	12	12
indole-3-butyric acid	ESI+	204→186	204→130	90	28	13
6-BA	ESI+	226→91	226→65	100	50	32
forchlorfenuron	ESI+	248→129	248→93	89	34	17
1-naphthylacetic methyl ester	ESI+	201→141	201→115	118	13	37

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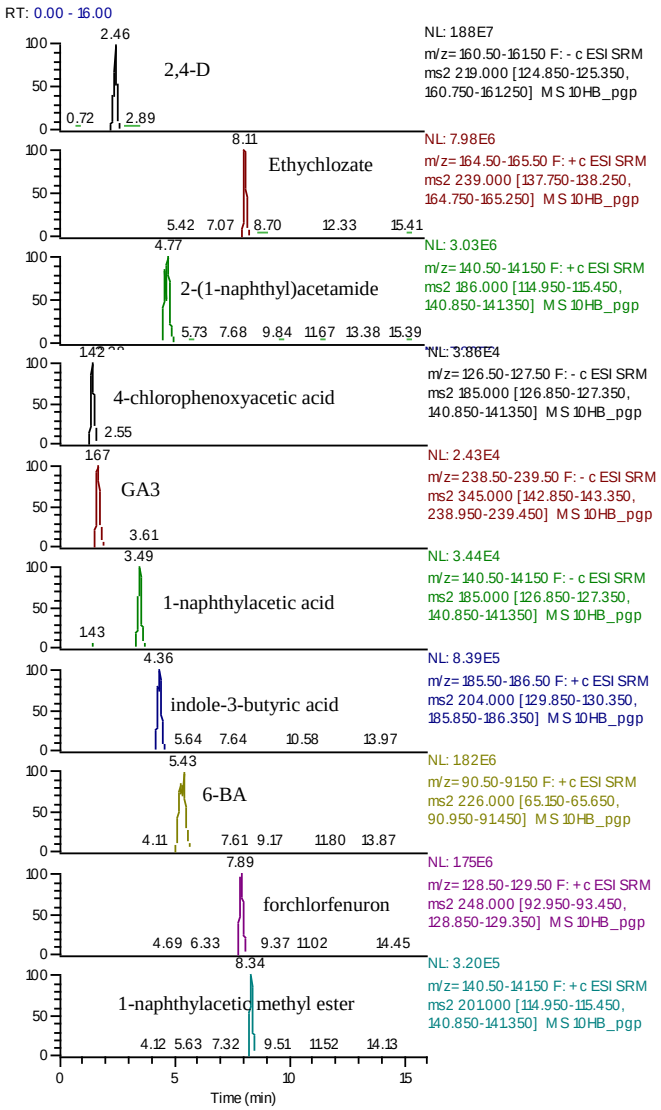

Fig. 1 The quantification MRM transitions of ten PGPs

QuEChERS method has been developed to different versions. In this study, the original QuEChERS method was minor modified as below: (a) adding 1% HAc to the MeCN for extraction; (b) using NaAc instead of NaCl[47-50]; (c) additional ultrasound extraction step; (4) the less amount of PSA.

To achieve high recoveries of both acidic and neutral target analytes, the pH value of the mobile phase was maintained at 4-5 by using Hac/NaAc buffer, which is naturally present in many fruits and can avoid potential analytical interferences or undesired effects. To investigate the effect of the extraction methods, ultrasound and vortex methods were performed, respectively. The results of two methods showed a negligible difference to sample A (containing high concentration of 1-naphthylacetic acid, above the LOQ), but showed a positive difference to sample B (with low concentration of 2,4-D, equally to its LODs), which meant both methods were not suitable for extracting low level of PGPs from real samples. Thereafter, the combination of shaking and ultrasonication was tried to enhance the extraction efficiency in our experiment, and good results were achieved. To avoid the accidence of interaction with the acid functionalities, the amount of PSA was decreased from 150 mg to 50 mg in this work to reduce the possible loss of acidic PGPs such as 2,4-D and GA3. Expectedly, the satisfied recoveries of them were reached.

3.3 Method validation

3.3.1 Matrix effect

The matrix effect is usually caused by matrix components in the extract. A higher matrix effect means lower accuracy and sensitivity of the method. Nowadays it is widely used to evaluate the performance of the sample pretreatment method. When the matrix effect is 100%, there is no matrix effect. And matrix effect values higher and lower than 100% correspond to suppression and enrichment of ionization by the matrix component, respectively. In this work, matrix

Table 2 Regression equations, correlation coefficients and linear ranges of PGPs in tomato matrix

Compound	Regression equations	linear ranges	correlation coefficients	LODs (μg·kg^{-1})	LOQs (μg·kg^{-1})
2,4-D	$Y=2.04\times10^{5}x+1725.71$	5~200	0.9993	1.5	5.0
4-chlorophenoxy-acetic acid	$Y=4.19\times10^{6}x+1.36\times10^{6}$	5~200	0.9969	0.4	1.3
GA3	$Y=9.31\times10^{5}x+1.01\times10^{6}$	5~200	0.9904	0.5	1.7
1-naphthylacetic acid	$Y=1.47\times10^{5}x+5.86\times10^{5}$	5~200	0.9970	1.0	3.3
Ethychlozate	$Y=3.03\times10^{5}x+4.40\times10^{5}$	5~200	0.9970	1.5	5.0
2-(1-Naphthyl)acetamide	$Y=2.28\times10^{6}x+7.22\times10^{6}$	5~200	0.9948	1.0	3.3
indole-3-butyric acid	$Y=9.00\times10^{4}x+2.43\times10^{5}$	5~200	0.9934	1.2	4.0
6-BA	$Y=3.18\times10^{5}x+9.00\times10^{4}$	5~200	0.9985	0.1	0.3
forchlorfenuron	$Y=1.75\times10^{5}x+5.35\times10^{4}$	5~200	0.9995	0.1	0.3
1-naphthylacetic methyl ester	$Y=8.74\times10^{3}x+3.52\times10^{4}$	5~200	0.9986	0.5	1.7

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Table 3 Recoveries, repeatabilities and reproducibilities in spiked concentrations in four fruits

analytes	Concentrations ($\mu g \cdot kg^{-1}$)	Watermelon R(%, n=6)			Grape R(%, n=6)			Cucumber R(%, n=6)			Tomato R(%, n=6)		
		R1*	R2*	R3*	R1	R2	R3	R1	R2	R3	R1	R2	R3
2,4-D	5	84.2	5.6	8.4	113.2	6.2	6.9	97.5	6.1	6.3	89.2	5.4	7.2
	20	87.5	8.4	8.1	91.1	5.1	6.1	96.6	4.4	5.2	99.8	6.1	5.9
	100	91.9	4.1	7.2	95.7	5.9	5.8	94.2	4.0	5.7	98.1	3.2	5.3
Ethychlozate	5	90.1	8.2	8.7	85.5	7.4	6.8	86.8	7.3	8.1	95.3	7.0	6.6
	20	95.5	4.1	6.5	93.7	5.0	5.4	97.2	3.4	8.0	90.9	4.2	6.1
	100	109.3	6.3	5.7	98.3	4.4	5.1	98.4	4.7	3.9	104.4	9.8	9.9
2-(1-naphthyl)acetamide	5	87.4	6.5	7.2	109.2	8.2	8.7	84.3	7.5	7.3	88.7	5.1	5.7
	20	89.2	5.4	5.1	93.7	5.9	6.7	88.9	5.9	6.2	92.9	4.9	6.1
	100	103.7	3.8	6.6	105.9	6.4	6.3	100.6	4.9	5.3	96.6	7.8	7.1
4-chlorophenoxy acetic acid	5	88.3	4.8	8.4	91.7	5.7	6.3	105.7	10.1	13.7	102.7	6.3	7.6
	20	91.1	3.9	7.1	86.8	6.6	6.2	97.6	7.1	8.2	80.8	9.3	14.7
	100	102.7	5.4	5.7	95.7	4.7	5.7	89.4	8.7	8.1	94.1	4.9	7.4
GA3	5	90.7	4.7	7.9	108.5	5.6	6.4	80.7	6.2	6.7	101.6	8.3	9.5
	20	86.9	7.5	7.1	91.8	6.9	7.5	85.2	5.8	6.1	95.7	7.1	7.9
	100	97.3	6.7	7.4	101.6	7.7	7.7	97.7	9.3	9.7	90.5	6.5	6.2
1-naphthylacetic acid	5	91.5	7.5	7.9	85.2	4.7	6.5	108.2	8.8	9.5	91.2	6.5	7.9
	20	96.7	5.7	6.3	92.6	5.1	6.3	91.4	8.2	7.8	94.5	7.1	7.4
	100	87.4	4.6	5.8	97.1	4.5	5.4	92.3	7.4	7.6	100.2	8.1	8.7
indole-3-butyric acid	5	85.7	7.5	6.2	89.4	7.3	9.1	80.5	8.9	9.5	86.8	8.4	7.6
	20	89.4	6.9	6.9	85.2	8.9	8.3	85.7	8.7	8.2	85.7	6.5	6.7
	100	98.7	6.2	7.1	95.1	7.0	7.4	91.5	7.8	7.1	99.1	7.7	7.1
6-BA	5	85.2	6.8	6.2	109.7	9.8	11.7	94.5	7.8	8.7	99.3	7.3	7.9
	20	96.1	5.5	6.3	99.4	6.6	6.1	91.2	8.4	8.1	93.2	8.9	8.3
	100	92.7	6.4	7.1	101.7	8.3	7.6	101.3	8.1	7.5	104.1	9.5	9.9
Forchlorfenuron	5	88.2	5.4	7.7	85.3	6.9	7.5	82.8	10.1	9.7	92.5	7.8	8.2
	20	107.4	6.7	6.2	95.8	7.6	7.1	101.2	9.5	12.1	98.3	8.4	9.1
	100	91.5	6.2	6.9	101.9	5.3	6.2	98.7	6.7	9.3	94.7	7.1	8.2
1-naphthylacetic methyl ester	5	83.2	7.9	7.4	105.8	7.9	9.2	90.2	6.1	5.7	97.2	7.1	7.6
	20	101.4	6.2	7.0	93.7	7.3	8.3	98.4	5.3	6.9	90.5	6.0	6.8
	100	94.9	6.3	6.0	97.2	7.5	6.4	99.9	5.8	5.2	93.3	7.6	7.3

Note: R1: recoveries; R2: repeatabilities; R3: reproducibilities

effect was studied by comparing the peak areas between standard solutions diluted by blank sample extract and organic solvent directly. The results exhibited that the matrix effect can be ignorable.

3.3.2 Analytical curve and linearity

Equations of calibration curves with relevant statistical parameters in tomato matrix were shown in table 2. It can be found that linear ranges were between 5.0 and 200.0 μg/kg with correlation coefficients (R^2) higher than 0.990 for all PGPs. For other three matrixes, cucumber, watermelon and apple, the similar results were obtained.

3.3.3 LODs and LOQs

Recently, Gupta et al.[21] developed a dispersive liquid-liquid microextraction method for simultaneous determination of different endogenetic plant growth regulators in common green seaweeds, and the results showed that the LODs of GA3 and indole-3-butyric acid were 1 μg/mL. Flores et al.[36] validated a QuEChERS-based extraction procedure for the multifamily analysis of phytohormones in vegetables by UHPLC-MS/MS, and the LODs were equal or higher than 2 μg/kg. Zhang et al.[37] analyzed four plant growth regulators in soybean sprouts and mung bean sprouts by QuEChERS-LC-MS/MS method, and the LODs of 2,4-D, GA3 and 6-BA were in the range of 0.27-9.3 μg/kg. Wang et al.[38] developed a simultaneous determination method of six regulators, using a mixed-mode functionalized calixarene as a solid-phase extraction sorbent, and the LODs of 2,4-D, 1-naphthylacetic

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Table 4 Concentration levels of ten target PGPs in fruit samples analyzed

Samples	Number of samples	Samples with PGP residues	Pesticide	Min. ($mg \cdot kg^{-1}$)	Max. ($mg \cdot kg^{-1}$)	MRL ($mg \cdot kg^{-1}$)
Tomato	20	10	1-naphthylacetic acid	5.1	106.8	100
			2,4-D	6.2	202.9	500
apple	10	3	1-naphthylacetic acid	1.5	45.7	100
			Ethychlozate	3.6	3.6	N*
cucumber	10	4	GA3	6.4	20.1	N
			forchlorfenuron	2.0	23.5	100
watermelon	10	3	forchlorfenuron	3.1	10.8	100

*N: No MRLs

acid and 6-BA were 20.4, 4.3 and 10.6 μg/kg, respectively. Yan et al.[51] prepared a new molecularly imprinted polymer for extracting four plant hormones followed by HPLC detection, and the LODs of indole-3-butyric acid and 1-naphthylacetic acid were 3.8 and 3.0 μg/kg, respectively. In this work, the LODs and LOQs ranged from 0.1 to 1.5 μg/kg and 0.3 to 5.0 μg/kg, respectively (As shown in Table 2). Compared with previous reports, the resulted LODs and LOQs of our method were much lower, which illustrated that our method could be more suitable for simultaneous analysis of various PGRs from different plant matrices. Lower LODs and LOQs demonstrated that the proposed method was much more sensitive.

3.3.4 Accuracy and precision

As shown in Table 3, satisfied method recoveries of 81−113% were obtained with RSD < 15% at fortification levels of 5, 20 and 100 $\mu g \cdot kg^{-1}$.

3.4 Analysis of real samples

The proposed method was further applied into the analyses of PGPs in real samples. Totally 50 samples, including 20 tomatoes, 10 cucumbers, 10 watermelons and 10 apples, were collected from local markets. To ensure the quality of the final results, the calibration curves were prepared daily in a blank matrix and between each batch of samples. In each batch of samples, a blank sample was spiked at LOQ level, extracted and analyzed. For the quality control compliance, the individual recovery of the spiked sample should be between 80 and 120%. After the analysis of the last sample in one batch, a point of the calibration curve was injected to verify that the calibration curve was still valid and that the response of the instrument was constant with a maximum admissible error of ±25%. The results were summarized in Table 4. It can be seen that there were 5 PGPs, 1-naphthylacetic acid, 2,4-D, Ethychlozate, GA3 and forchlorfenuron, detected in real samples.

4. Conclusions

In conclusion, in the present study, a rapid and sensitive multi-residue method for the simultaneous determination of ten PGPs in fruit and vegetable samples has been introduced by the combination of a modified QuEChERS method and LC-MS/MS with a negative-to-positive ionization switch mode. The proposed method showed high recovery and accuracy for all target PGPs, and can meet all the requirements of European regulations regarding the analysis of pesticide residues.

Acknowledgments

We gratefully acknowledge General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (Scientific research plan project, 2011QK385) for funding this project.

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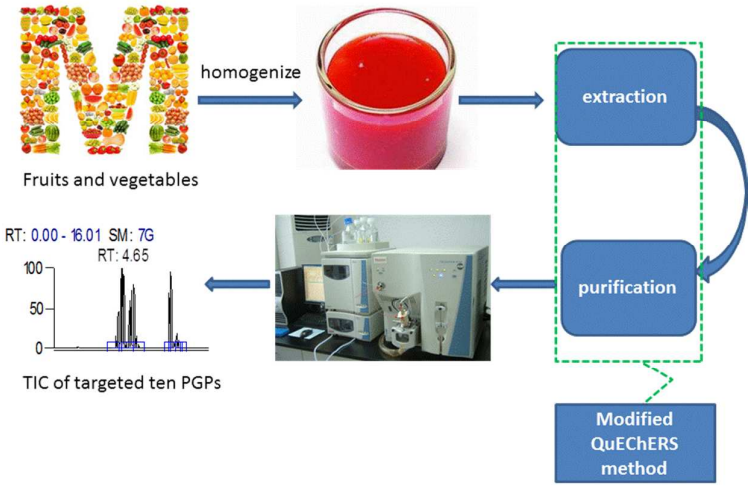
A graphical and textual abstract



In this study, the QuEChERS methodology coupled with MS triple quadrupole was used to determine 10 plant growth promoters (PGPs) popularly used in fruits and vegetables, and the results demonstrated that the developed method offers fast and safe alternative to typical multi-PGPs analysis methods for real samples.