Determination of Ten Plant Growth Regulator Residues in Bean Sprouts by Mass Spectrometry

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Abstract [**Objectives**] This study was conducted to quickly qualitatively and quantitatively analyze the residues of 10 plant growth regulators (PGRs) in bean sprouts. [**Methods**] Using bean sprouts as the test material, a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was established to determine the residual levels of 10 PGRs in bean sprouts. [**Results**] Under optimized conditions, the retention time of the 10 PGRs ranged from 6.45 to 11.43 min. When the mass concentration ranged from 0.005 to 0.05 μg/ml, all PGRs exhibited good linearity, with correlation coefficients (r) ≥0.999 1. The limits of detection (LODs, $S/N=3$) were in the range of 0.30 -0.92 μg/kg, and the limits of quantification (LOQs) were in the range of 0.50 -2.10 μg/kg. The average recovery values at three concentration levels ranged from 80% to 105.8%, with relative standard deviations (*RSD*s, $n=6$) of 2.8% -7.5%. [**Conclusions**] This method is simple and accurate, and provides technical reference for food safety monitoring.

Key words HPLC-MS/MS; Bean sprout; Plant growth regulators; Mass spectrometry

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Bean sprouts, also known as a kind of "sprouting vegetable" or "living vegetable", are highly nutritious, rich in vitamins, minerals, free amino acids, and other components beneficial to human health. In recent years, incidents of "toxic bean sprouts" have frequently occurred, with reports of plant growth regulators, fungicides and even antibiotics being detected in bean sprouts, posing a serious threat to consumer health. Plant growth regulators (PGRs) can promote bud formation and inhibit root growth in plants. Fungicides and pesticides are used to kill bacteria, molds, and pests in bean sprouts. Antibiotics prevent root rot, thereby preserving freshness. Long-term or excessive consumption of such "toxic bean sprouts" may lead to liver damage, allergic reactions, or induce pathogen resistance[1-3].

In recent years, food safety incidents caused by excessive and indiscriminate use of PGRs have occurred frequently, raising widespread concerns about their safety and residue issues[4-7]. The GB 2763-2016 *National Food Safety Standard—Maximum Residue Limits for Pesticides in Food* specifies the maximum residue limits (MRLs) for certain PGRs including 2,4-dichlorophenoxyacetic acid (2,4-D), forchlorfenuron (CPPU), thidiazuron (TDZ), and paclobutrazol (PBZ), the ranges of which were set as 0.01 -2.00, 0.05 -0.10, 0.05 -1.00 and 0.2 -0.5 mg/kg, respectively. In 2015, China Food and Drug Administration (CFDA), the Ministry of Agriculture and other departments jointly issued a ban on the use of PGRs such as 6-benzyladenine (6-BA) and 4-chlorophenoxyacetic acid (4-CPA) in bean sprout production. However, some producers still illegally add chemicals like "AB powder" (where powder A contains 6-BA and powder B contains gibberellin (GA_3) and "rootless bean sprout hormone" (containing 4-CPA) to maximize profits. Additionally, substances such as indole-3-acetic acid (IAA) and 4-fluorophenoxyacetic acid have also been illicitly used in bean sprout production[8-10]. To effectively monitor the residues of PGRs in bean sprouts, it is essential to establish efficient and rapid detection methods to provide technical support for food safety supervision.

Materials and Methods

Instruments and equipment

Agilent 6460 HPLC-MS/MS (Agilent Technologies (China) Co., Ltd.); 099ADPM12 vortex mixer (Shanghai Equl Corporation); 3 -15 high-speed centrifuge (Hettich, Germany); EVA50A nitrogen evaporator (Organomation Associates, USA); T-200 electronic balance (Changshu Shuangjie Instrument Factory); SZ13022-NY 0.22 μm organic filter membrane (Tianjin Linghang Experimental Equipment Co., Ltd.).

Standards of 10 PGRs: Abscisic acid, indolepropionic acid, indole-3-acetic acid, 4-chlorophenoxyacetic acid, thidiazuron, 6-benzylaminopurine, 4-iodophenoxyacetic acid, triapenthenol, 2,4-D, and 1-naphthaleneacetic acid (purity ≥99.0%, National Standard Material Center); methanol, formic acid, acetonitrile, NH_4AC (HPLC grade, ASC Inc., USA); anhydrous magnesium

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sulfate, sodium chloride, acetic acid (analytical grade, Sinopharm Chemical Reagent Co., Ltd., Shanghai).

Experimental methods

Chromatographic conditions Column: Phenomenex H18 (50 mm × 3.00 mm, 2.60 μm); injection volume: 5 μl; column temperature: 35 ℃; mobile phase: A (5 mmol/L ammonium acetate containing 0.1% formic acid) and B (methanol). Gradient elution program: 0.5 – 5.0 min, 10% B; 5.0 – 8.0 min, 95% B; 8.0 – 12.0 min, 10% B; flow rate: 0.25 ml/min.

MS conditions Electrospray ionization (ESI) source, positive (ESI^+) and negative (ESI^-) ion scanning modes; nebulizing gas flow rate 3.00 L/min; drying gas flow rate 15.00 L/min; DL tube temperature 250 ℃; electrospray voltage (IS): 2 500 V; scanning time 0.1 s; multiple reaction monitoring (MRM) mode.

Preparation of standard solutions A 100 μl aliquot of each PGR standard solution (1 000 μg/ml) was precisely transferred and diluted with methanol to 10 ml to obtain a mixed standard stock solution with a concentration of 10 μg/ml, which was stored at 4 ℃. Matrix-mixed standard working solutions at concentrations of 0.005, 0.01, 0.02, 0.04, and 0.05 μg/ml were prepared using blank sample solutions and used immediately after preparation.

Sample pretreatment A 5.0 g of bean sprout sample was weighed into the outer tube of a centrifuge tube. The mixed standard solution (external standard method) was added and allowed to stand for 30 min. Then, 10 ml of 5% acetic acid-acetonitrile (1 : 99, *v/v*) was added along with an extraction salt packet (containing 5.5 g of anhydrous magnesium sulfate, 1.5 g of sodium chloride, 0.5 g of disodium hydrogen citrate, and 1.0 g of trisodium citrate) and 10 zirconium beads. The inner tube (containing five 4-mm zirconium oxide beads and 500 mg of anhydrous magnesium sulfate) was tightened. After 25 min of alternated oscillation and centrifugation, 1.0 ml of supernatant was accurately collected, evaporated to near dryness under nitrogen, and reconstituted with 10% methanol solution. The solution was filtered through a 0.22 μm organic microporous membrane, and the obtained filtrate was analyzed by HPLC-MS/MS.

Results and Analysis

Optimization of MS conditions

A 1 000 μg/L standard solution of each of the 10 PGRs was individually infused into the MS system via a syringe pump for parameter optimization. Full scans were performed in both positive and negative ion modes, with a scan range of *m/z* 50 – 500, to obtain the primary mass spectra of the 10 PGRs. Due to differences in the properties of the 10 PGRs, their ionization modes also varied. Therefore, the appropriate ionization mode for each PGR was determined to achieve optimal ionization efficiency. The ion with the highest response was selected as the precursor ion, and the declustering voltage (DP) was optimized to obtain the strongest parent ion signal. Two ions with relatively high responses were chosen as the quantitative and qualitative ions, respectively. In multiple reaction monitoring (MRM) mode, the collision energy (CE) was optimized. The MS parameters are listed in Table 1.

Table 1 MS parameters of the 10 PGRs

Compound	Ionization mode	Mass-to-charge ratio // *m/z*		Declustering voltage // V	Collision energy // eV	Retention time // min
		Parent ion	Daughter ion			
6-Benzylaminopurine	ESI^-	224.0	133.0 *, 115.8	30	16, 25	6.45
Abscisic acid	ESI^-	264.4	155.4 *, 220.7	17	15, 20	6.89
Indolepropionic acid	ESI^-	188.1	69.5 *, 114.2	25	18, 30	7.15
4-Chlorophenoxyacetic acid	ESI^-	185.0	127.1 *, 141.9	15	19, 26	7.69
Thidiazuron	ESI^-	220.8	105.4 *, 79.5	14	16, 20	7.98
Indole-3-acetic acid	ESI^-	202.5	118.4 *, 160.7	23	15, 22	8.15
4-Iodophenoxyacetic acid	ESI^-	278.9	220.1 *, 138.9	19	17, 23	8.73
2,4-Dichlorophenoxyacetic acid	ESI^-	218.9	160.6 *, 125.1	15	18, 28	9.20
1-Naphthaleneacetic acid	ESI^-	182.4	140.2 *, 123.8	12	10, 17	9.39
Triapenthenol	ESI^+	330.4	225.3 *, 118.9	18	15, 26	10.43

* indicates quantitative ions.

Methodology validation

Linear ranges, limits of detection (LODs) and limits of quantitation (LOQs) Matrix-mixed standard solutions at concentrations of 0.005, 0.01, 0.02, 0.04 and 0.05 μg/ml were prepared. Standard curves were plotted with the mass concentration as the *x*-axis and the peak area as the *y*-axis. The linear regression equations and correlation coefficients are presented in Table 2. The results demonstrated that the 10 PGRs exhibited good linearity within the concentration range of 0.005 – 0.05 μg/ml, with correlation coefficients (*r*) all ≥0.999 1. The LODs were calculated based on a signal-to-noise ratio (*S/N*) of 3, while the LOQs were determined at *S/N* = 10. The LODs for the 10 PGRs ranged from 0.03 to 0.92 μg/kg, and the LOQs ranged from 0.50 to 2.10 μg/kg (Table 2).

Method recovery and precision Blank bean sprout samples (5.0 g) free of the target analytes were spiked with the 10 PGR standards at three concentration levels (5, 50 and 100 μg/kg), with three replicates per level. Each concentration was determined for six times repeatedly, and the relative standard deviation (*RSD*) of the measured values was calculated. As shown in Table 3, the

average recovery values at all spiked levels ranged from 80% to 105.8%, with *RSD*s between 2.8% and 7.5%. The method demonstrated satisfactory accuracy and precision, meeting the requirements for residue analysis.

Table 2 Linear ranges, correlation coefficients, LODs and LOQs of the 10 PGRs

Compound	Equation of linear regression	Correlation coefficient (r)	LOD//μg/kg	LOQ//μg/kg
6-Benzylaminopurine	$y=5.215x+1.023$	0.999 2	0.50	1.72
Abscisic acid	$y=10.287x-1.054$	0.999 4	0.83	1.69
Indolepropionic acid	$y=9.348x+0.267$	0.999 3	0.11	0.72
4-Chlorophenoxyacetic acid	$y=6.358x-1.367$	0.999 1	0.75	1.16
Thidiazuron	$y=11.578x+1.488$	0.999 7	0.03	0.50
Indole-3-acetic acid	$y=5.397x+1.024$	0.999 6	0.45	0.73
4-Iodophenoxyacetic acid	$y=12.572x-2.245$	0.999 3	0.89	1.89
2,4-Dichlorophenoxyacetic acid	$y=11.025x+1.981$	0.999 1	0.09	0.61
1-Naphthaleneacetic acid	$y=9.157x-2.019$	0.999 3	0.07	0.58
Triapenthenol	$y=13.547x+1.087$	0.999 1	0.92	2.10

Table 3 Recovery and precision of 10 PGRs ($n=6$) %

Compound	Spiking level 5.0 μg/kg		Spiking level 50.0 μg/kg		Spiking level 100.0 μg/kg	
	Recovery	*RSD*	Recovery	*RSD*	Recovery	*RSD*
6-Benzylaminopurine	86.4	5.8	92.8	4.7	104.2	3.1
Abscisic acid	81.6	6.8	86.0	5.3	95.7	4.7
Indolepropionic acid	80.0	7.3	85.7	6.0	91.8	4.2
4-Chlorophenoxyacetic acid	85.7	6.9	90.1	4.1	105.8	3.8
Thidiazuron	80.9	7.5	86.4	5.8	93.6	5.0
Indole-3-acetic acid	86.7	6.1	93.8	5.1	98.9	2.8
4-Iodophenoxyacetic acid	82.7	6.8	88.7	4.6	93.3	3.7
2,4-Dichlorophenoxyacetic acid	86.3	7.2	93.8	5.9	104.7	3.4
1-Naphthaleneacetic acid	85.0	6.8	92.0	6.1	97.8	4.1
Triapenthenol	85.2	7.4	89.7	5.3	93.5	4.3

Analysis of actual samples

The established analytical method was applied to test 30 randomly purchased batches of bean sprout samples from the market. Among the inspected samples, 4-chlorophenoxyacetic acid (4-CPA) was detected in 2 batches, with contents below the limit of quantification, and 6-benzyladenine (6-BA) was detected in 1 batch, with contents of 2.1 and 3.5 μg/kg, respectively, making them unqualified samples.

Conclusions and Discussion

This study optimized the MS conditions and established an HPLC-MS/MS method for the simultaneous determination of 10 PGRs in bean sprouts. The linear ranges, LODs, LOQs, recovery and precision of the method were validated, and its practical application effectiveness was analyzed. The method has the advantages of simple operation, high sensitivity and good reproducibility, and meets methodological requirements in terms of accuracy and precision, making it suitable for the simultaneous determination of residual levels of 10 PGRs in bean sprouts.

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shape with purple-red skin and flesh. The flesh is fine-textured, and crisp. The fleshy roots are rich in anthocyanins, and slightly pungency under high temperatures, and have excellent toughness. The variety exhibits high commodity rate and outstanding uniformity.

Fig. 1 A new fruit radish variety 'Sheng Cui 745'

Key cultivation techniques

This variety is suitable for open-field autumn cultivation in Shandong, Tianjin, Hebei and similar regions. The optimal sowing period falls between late August and early September. Generally, 2 seeds are sown per hole, and the seeding rate is 1 500 – 2 250 g/hm^2. After sowing, the seeds are covered with 0.5 cm of fine soil. When seedlings develop 5 – 6 true leaves, and the fleshy roots begin to expand (known as 'root breaking' stage), final singling is performed according to specified plant spacing, and intertillage weeding and thinning are performed simultaneously. During the peak expansion period of fleshy roots, the water demand reaches its maximum, and thorough and even irrigation is required while maintaining soil available water content above 70% – 80% to prevent hollowness and root cracking simultaneously. The optimal harvest period occurs 65 – 70 d after sowing when the roots reach full expansion but before frost exposure, as premature harvesting affects yield. Timely harvest after complete root expansion ensures maximum yield, and the roots should be washed for sales or cold storage.

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