APPLICATION NOTE

Liquid Chromatography
Mass Spectrometry

Authors:

Cheng-Yuan Cai
Li-Zhong Yang
Yong-Ming Xie

PerkinElmer, Inc.
Shanghai, China

Feng Qin

PerkinElmer, Inc.
Canada

Simultaneous Determination of Plant Growth Regulators in Bean Sprouts by Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry

Introduction

Bean sprouts are a commonly used ingredient in asian cuisine and highly valued for their nutritional properties. In recent years there have been concerns about the presence of exogenous plant growth regulators in bean sprouts[1,2], which can accelerate their growth. Though this accelerated growth is great for production, the addition of growth regulators is known to be harmful to the health of consumers.

To effectively control the residues of these compounds in food, the Ministry of Agriculture of China has banned the use of gibberellin, benzyladenine and 4-chlorophenoxyacetate in bean sprouts since 2015. Thus, it is of great importance to develop a simple and sensitive method for the simultaneous determination of plant growth regulators in bean sprouts.

PerkinElmer®
For the Better

Experimental:

Sample Treatment: All samples were obtained from a local market. After being homogenized, 2.5 g of sample was weighed into a centrifuge tube. 5 mL acetonitrile and 1.5 g of NaCl was added to the tube, which was then mixed for one minute and placed in an ultrasonic bath for five minutes. The tube was then centrifuged at 6000 rpm for five minutes. The final solution was diluted five-fold with water and filtered through a 0.22 µm membrane filter.

Standard calibration solutions: Stock solutions (1 mg/mL) of analytical standards were prepared by dissolving 10 mg of the compounds in 10 mL of methanol. The standard working solutions were prepared by serial dilution (0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 ng/ml) of the stock solution with water–acetonitrile (80:20, v/v).

LC Conditions: The analytes were separated on a PerkinElmer Altus® A-30 UPLC® system using a PerkinElmer SPP C18 column (2.1 x 100 mm, 2.7 µm). The temperature of the column oven was set at 40 °C. The mobile phase consisted of water containing 0.01% formic acid (A) and acetonitrile (B). The flow rate was 0.3 mL/min and the elution gradient is shown in Table 1. The injection volume was 10 µL.

MS Conditions: The Altus UPLC® system was coupled to a QSight™ 210 triple quadrupole mass spectrometer equipped with an electrospray ionization source operating in negative ion mode. The mass spectrometer operating conditions were as follows: ElectroSpray: -5000 v, Heating Gas Temp: 500 °C, HSID Temp: 320 °C, Dry Gas: 70, Nebulizer Gas: 160. Detection of analytes by tandem mass spectrometry was conducted in multiple reaction monitoring mode (MRM). A summary of the monitored ions and the optimized MS and MS/MS parameters for the analytes is shown in Table 2. The MRM dwell time was set at 30 ms for each transition.

Data aquisition and processing was performed using the PerkinElmer Simplicity 3Q™ software.

Validation was conducted by characterizing method specificity, matrix effect sensitivity, linearity, accuracy, and precision.

Table 1. LC eluent gradient.

	Time (min)	%A	%B	Flow rate (ml/min)
1	0.0	95.0	5.0	0.3
2	0.3	90.0	10.0	0.3
3	5.0	45.0	55.0	0.3
4	5.5	5.0	95.0	0.3
5	6.5	5.0	95.0	0.3
6	6.6	95.0	5.0	0.3
7	9.0	95.0	5.0	0.3

Table 2. Target analytes and MRM transitions.

Analyte	Formula	CAS	MRM
4-chlorophenoxyacetate	C8H7ClO3	13730-98-8	184.7/126.8; 186.6/128.8
Benzyladenine	C12H11N5	1214-39-7	223.8/133.0; 233.8/131.9
Gibberellin	C19H22O6	77-06-5	344.8/142.9; 344.8/238.9
Forchlorfenuron	C12H10ClN3O	68157-60-8	245.7/126.8; 247.7/128.8
2,4-D	C8H6Cl2O3	94-75-7	218.7/160.7; 220.7/162.7
3-Indolebutyric acid	C12H13NO2	133-32-4	201.6/116.1; 201.6/157.8

Results and discussion

Specificity of analysis was tested by spiking trace level concentration of a mix of growth hormones into bean sprout matrix and matched against the blank matrix (Figure 1).

The results suggested there was no matrix interference and the method could be successfully applied to samples with no additional sample clean up.

To evaluate ion suppression from matrix effects, the signal intensity of the standard solution (A) was compared with that of the matrix-matched standard solution (B) at different concentrations. A negative matric effect (B/A × 100%) < 100% indicates matrix suppression, whereas a positive one (> 100%) indicates ionization enhancement. Figure 2 shows examples of signal intensities in standard solution and matrix matched standard solution.

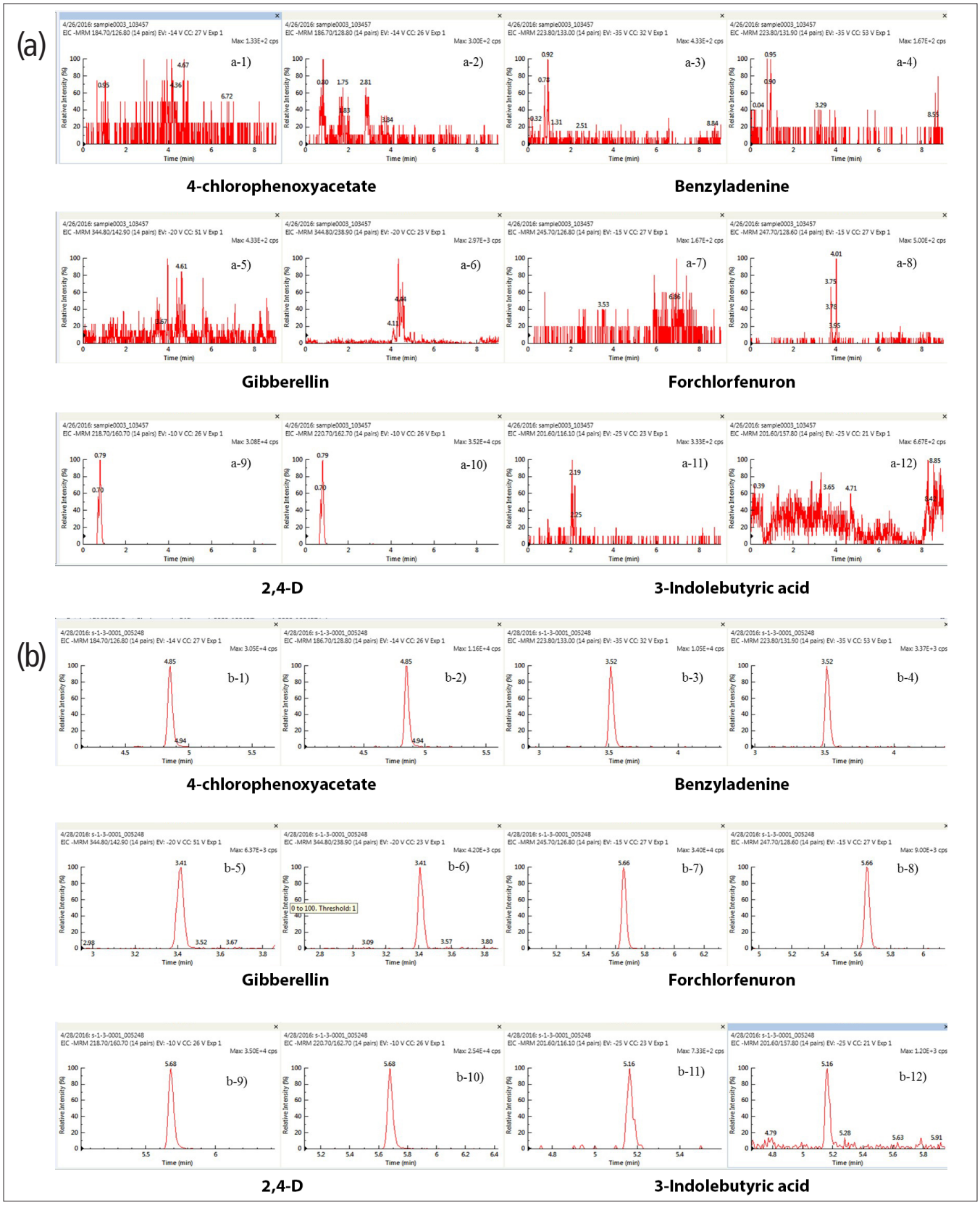

Figure 1. Typical chromatograms of bean sprouts as acquired by MRM: (a) blank sample and (b) spiked with 1 μg/kg with analytes.

The matrix effect was calculated for all six compounds in mung bean sprouts and soybean sprouts and listed in Table 3. Except for 3-indolebutyric acid, the five other analytes showed no significant matrix effect. 3-indolebutyric acid showed strong matrix suppression. In this study, matrix-matched calibration curves were used for quantification to compensate for any matrix effect during the experiment.

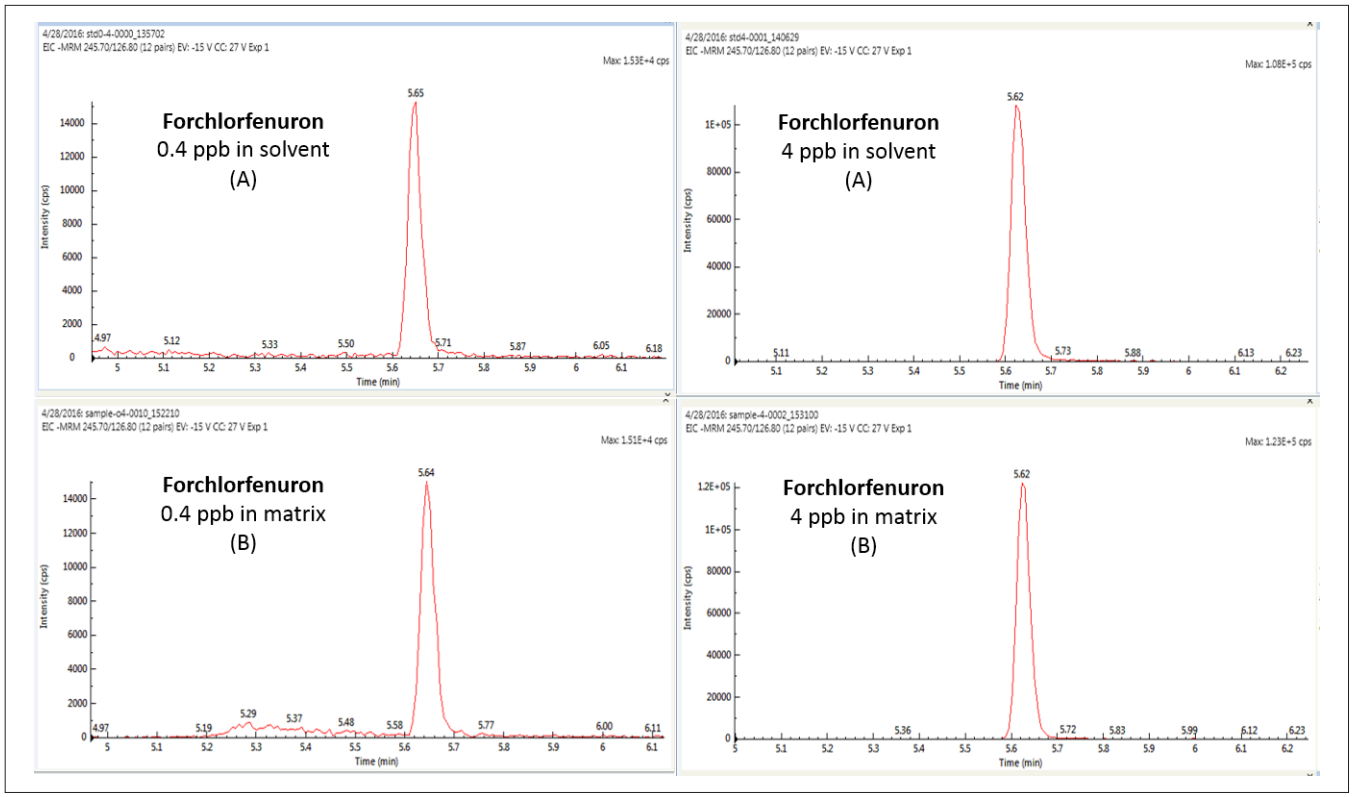

Figure 2. Example of signal intensities in standard solution (A) and matrix matched standard solution (B)

Recovery data for analytes spiked in matrices are summarized in Table 4. Overall, recoveries were between 66% and 104%.

Calibration was evaluated by matrix-matched calibration standards prepared as described in the experimental section. Figure 3 shows the calibration curve for 4-chlorophenoxyacetate in bean sprout matrix. Table 5 presents the linear dynamic range and limits of quantification (LOQ) values for this method. The correlation coefficients (R^2) were all above 0.990.

Table 3. Matrix effect for target analytes in mung and soybean sprouts.

	mung bean sprout	soybean sprouts
4-chlorophenoxyacetate	102%	104%
Benzyladenine	99%	95%
Gibberellin	107%	115%
Forchlorfenuron	119%	113%
2,4-D	108%	101%
3-Indolebutyric acid	71%	60%

Table 4. Analyte recoveries for spiked matrices.

	Spiked level (0.4ppb)(%)	Spiked level (1 ppb)(%)	Spiked level (4 ppb)(%)
4-chloro phenoxyacetate	66	71	69
Benzyladenine	71	73	72
Gbberellin	66	69	66
Forchlorfenuron	97	94	104
2,4-D	68	74	70
3-Indolebutyric acid	78	72	75

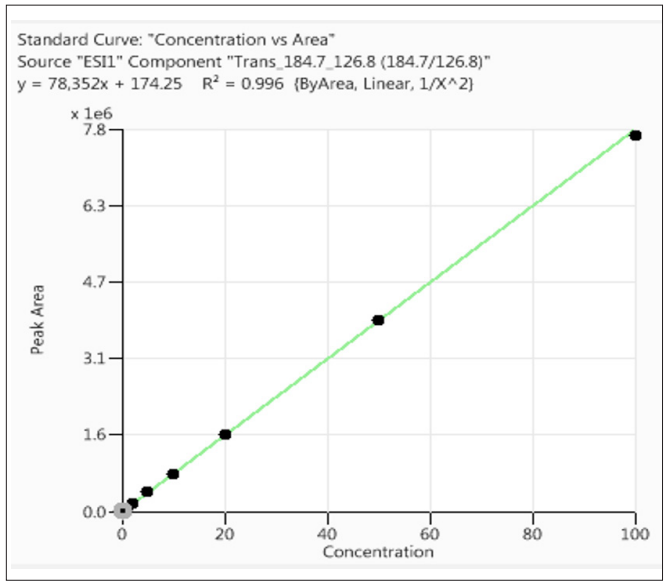

Figure 3. calibration curves for 4-chlorophenoxyacetate compounds in bean sprout matrix (Linear range: 0.005-100 ng/ml, R^2>0.990, >4.5 order, accuracy<15%).

Table 5 Linear range, linearity and LOQ values

	Linear Range (ng/mL)	R^2	LOQ (ug/kg)
4-chloro phenoxyacetate	0.005-100	0.996	0.05
Benzyladenine	0.02-100	0.991	0.2
Gibberellin	0.02-100	0.995	0.2
Forchlorfenuron	0.005-5	0.992	0.05
2,4-D	0.01-50	0.991	0.1
3-Indolebutyric acid	0.2-100	0.992	2

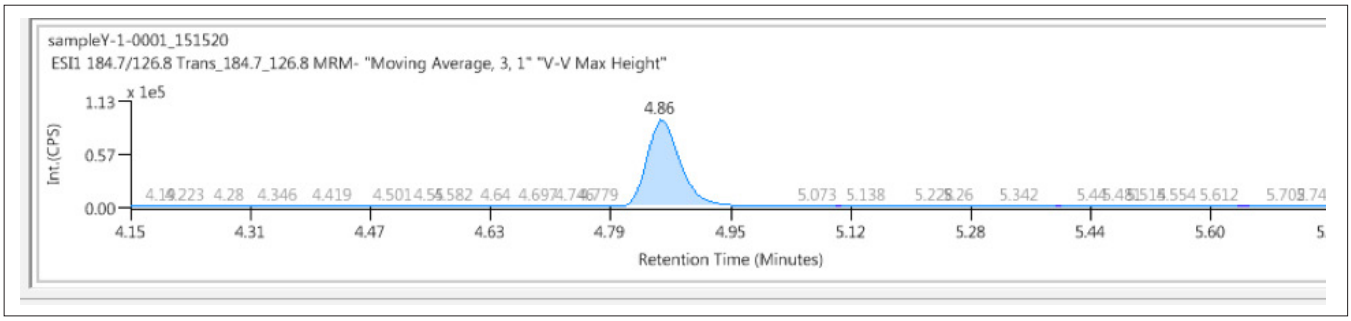

Figure 4. Typical chromatograms of real sample containing 4-chlorophenoxyacetate.

Table 6. Results summary of analyte content in actual samples, labeled Y1-Y8 and SH1-SH2 (units: ug/kg)

	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	SH1	SH2
*4-chlorophenoxyacetate	37.11	NA	NA	26.85	NA	NA	NA	NA	35.50	18.13
*Benzyladenine	1.61	4.43	4.61	1.44	48.41	9.82	49.76	NA	2.73	59.1
*Gibberellin	NA	NA	NA	NA	5.38	NA	NA	NA	NA	NA
Forchlorfenuron	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2,4-D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3-Indolebutyric acid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

The developed method was applied to analysis of 10 samples obtained from different local markets. Typical chromatograms of a contaminated sample are shown in Figure 4. The results for all analyzed samples are summarized in Table 6.

The method was in compliance with the requirements set by Document No. 1 of 2003, dispatched by Ministry of Agriculture of China.

Conclusions

We have developed a rapid, sensitive, and reproducible LC/MS/MS method for the analysis of six plant growth regulators in bean sprouts, using a simple sample preparation procedure. The LOQ for all analytes were within 0.05-2 µg/kg. The QSight mass spectrometer provides a robust platform for analysis of trace level plant growth regulators and can easily meet limits set by the Chinese regulatory bodies.

References

1. Soon-Kil Cho, A.M. Abd El-Aty, Ki Hun Park et al. Food Chemistry. 136 (2013) 1414–1420.
2. Liyan Ma, Hongyan Zhang, Wentao Xu. Food Anal. Methods (2013) 6:941–951.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013027_01 PKI