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Determination of Diacylhydrazines-Type Insect Growth Regulator JS-118 Residues in Cabbage and Soil by High Performance Liquid Chromatography with DAD Detection

J.-Y. Hu · Z.-B. Deng · D.-M. Qin

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Abstract JS-118 is a diacylhydrazines-type insect growth regulator used extensively in China now. An analytical method for residues determination of JS-118 in cabbage and soil samples by high performance liquid chromatography with DAD detection was established and optimized. Primary secondary amine solid phase extraction cartridge was used for sample preparation. Mean recoveries for the analyte ranged from 96.6% to 107.0% with CV value less than 4.7%. The limit of quantification is 0.01 mg/kg. Direct confirmation of JS-118 residues in samples was realized by high performance liquid chromatography-mass spectrometry. The proposed method is simple, rapid and reliable to perform and could be utilized for monitoring of pesticides residues.

Keywords JS-118 · Residues determination · Solid phase extraction · HPLC-DAD

The insecticide JS-118 [N′-t-Butyl-N′-(3, 5-dimethylbenzoyl)-(2,7-dimethyl-benzodihydrofuran) -methylbenzohydrazine], [CAS NO. 467427-81-1] is a recently introduced diacylhydrazines-type insect growth regulator to control *Lepidoptera* (Zhang 2005). It was developed by Jiangsu Pesticide Research Institute China in 1990s, has been granted patents in China (ZL01181611.9). Our previous work has studied the hydrolysis and photolysis of JS-118 in aqueous solutions under abiotic conditions (Hu et al. 2009).To our knowledge no residue analytical methods are published in literatures to determine JS-118 residues in vegetable and soil samples, although it has been marketed in China for several years. In this paper an HPLC-DAD method combined with solid phase extraction to determine JS-118 residues in cabbage and soil is described, the average recoveries were excellent and the limit of quantification in the samples of the method was 0.01 mg/kg. JS-118 residues in the samples were further confirmed by high performance liquid chromatography mass spectrometry (LC-MS) Fig. 1.

J.-Y. Hu (✉) · Z.-B. Deng
School of Applied Science, University of Science and Technology Beijing, 100083 Beijing, China
e-mail: jiyehu@sas.ustb.edu.cn

D.-M. Qin
Institute for the Control of Agrochemicals, Ministry of Agriculture, 100026 Beijing, China

Materials and Methods

JS-118 standard (purity, 99.5%) was obtained from Jiangsu Pesticide Research Institute China. HPLC grade acetonitrile was procured from Dikma Limited (China). Other solvents and chemicals used were of analytical grade from Dikma Limited (China). SPE columns were Dikma Limited Sample Preparation Products (500 mg, 3 mL). The samples of cabbage and soil were obtained from farms that use only pesticide-free agriculture to raise the crops.

High Performance Liquid Chromatograph (SHIMADZU) equipped with an analytical column (250 × 4.5 mm I.D., 5 μm ODS) attached to a DAD detector. The chromatographic conditions used for the analysis of JS-118 residues were as follows: For the analysis of cabbage samples, the mobile phase was acetonitrile-water (70:30, v/v), and for the analysis of soil samples, the mobile phase was acetonitrile-water (65:35, v/v).The total flow was 1.0 mL/min. The injection volume was 20 μL; Detection was performed at 230 nm. Under these conditions, the retention time of JS-118 was about 7.3 min for cabbage samples and 9.5 min for soil samples. All measurements were carried out at room temperature.

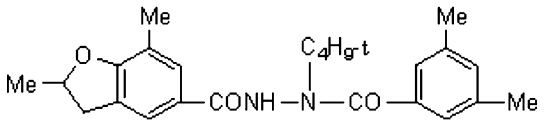

Fig. 1 Chemical structure of JS-118

Shimadzu LCMS-2010EV system was employed. Acquisition parameters were as follows: Column, Shimadzu XR-ODS, 2.0 × 75 mm, 2.2 μm. Flow rate, 0.4 mL/min. Temperature, 40°C. Mobile phase, A—water; B—acetonitrile, A:B = 30:70. Mass Detector parameters: ESI (+); Nebulizing gas: 1.5 L/min; Drying gas: 0.1 MPa; Block Heater: 200°C; Detector Voltage: 1.6 Kv; Scan Range: m/z, 100–1000.

Standard solution (1,000 mg/L) of JS-118 were prepared in a mixture of methanol and water (7/3, v/v).The solutions required for preparing a standard curve (0.05, 0.1, 0.5, 1, 5 and 10 μg/mL) were prepared from the stock solution by serial dilutions. All solutions were protected against light with brown container and were stored in a refrigerator at 4°C.

Cabbage Sample

Twenty gram aliquot of chopped and homogenized samples was weighed in a 250 mL polypropylene container; sodium chloride (5 g) and acetonitrile (50 mL) were added. The mixture was vigorously shaken for 30 min, and filtered through a 12 cm Buchner funnel; the solid residues were treated with an additional acetonitrile 10 mL). The filtrate was transferred into a 500 mL separatory funnel, and the phase was allowed to separate by salting out process. The lower aqueous layer was discarded and the upper organic layer was collected through the common funnel filled with anhydrous sodium sulfate (5 g) in order to remove the residue water. The dried extract was evaporated at 40°C to dryness on the rotary evaporator. The residue obtained was dissolved in 3 mL *n*-hexane-dichloromethane (8/2, v/v). Extract purification was performed on PSA cartridges. PSA cartridges were preconditioned with the mixture of *n*-hexane-dichloromethane (8/2, v/v). The cartridges were loaded with 2 mL *n*-hexane-dichloromethane extract above. This eluate (2 mL) was discarded. The cartridges were re-washed with 2 mL *n*-hexane-dichloromethane (8/2, v/v), and discarded. Analyte JS-118 was eluted with 2 mL *n*-hexane-dichloromethane (6/4,v/v) and concentrated in a rotary evaporator (35°C) and completely dried under a nitrogen purge. The residue was re-dissolved in 2 mL of the mobile phase for HPLC analysis.

Soil Sample

Soil samples (20 g, passed through a 2 mm sieve) were extracted by ultrasonic extraction for 30 min with a mixture of 60 mL methanol-water (1/1, v/v), and filtered through a 12 cm Buchner funnel; the solid residues was treated with an additional mixture of 10 mL methanol-water (1/1, v/v).The combined extracts were transferred to a separatory funnel (500 mL) with 30 mL 2% Na_2SO_4 aqueous solutions and then 60 mL of dichloromethane as the rinse. After the mixture was shaken for 2 min, the dichloromethane layer was collected, and the aqueous layer was extracted two more times, using 20 mL of dichloromethane each time. The combined dichloromethane extract was dried over anhydrous sodium sulfate (2 cm bed), followed by the removal of the solvent in a vacuum rotary evaporator at 40°C. The residue of the extracts was dissolved with 3 mL *n*-hexane-dichloromethane (8/2, v/v) and for purification by PSA cartridges followed the same procedures of above cabbage samples.

Recovery experiments were carried out, in three replicates, at three fortification levels (0.01, 0.1 and 0.5 mg/kg) by adding known volumes of pesticide standards solutions to different matrices (cabbage and soil). Blank analyses were performed in order to check interference from the matrix.

Results and Discussion

Standard calibration curve of JS-118 was constructed by plotting the analyte concentration against peak areas. At

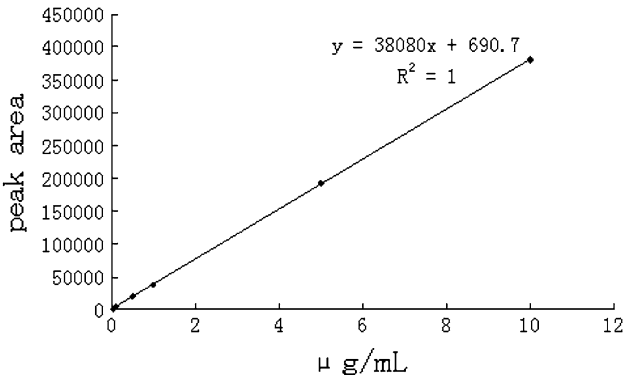

Fig. 2 Linearity correlation

Table 1 Recoveries of JS-118 residues in cabbage and soil a (n = 3)

Samples	Fortification levels (mg /kg)	Average recoveries (%)	CV (%)
Cabbage	0.01	107.0	2.6
	0.1	95.6	0.4
	0.5	98.9	0.5
Soil	0.01	105.5	0.7
	0.1	102.5	2.1
	0.5	104.5	4.7

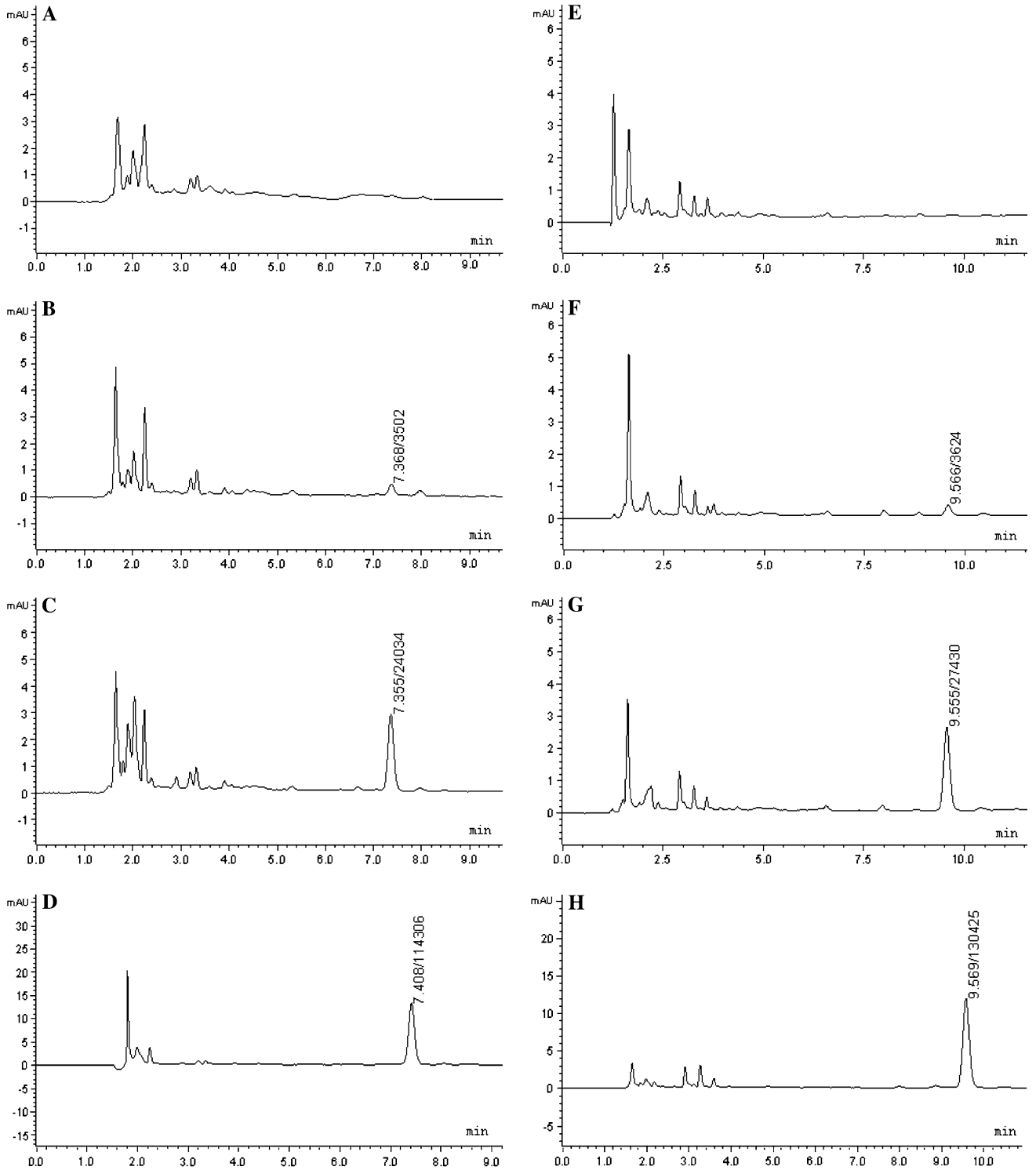

Fig. 3 The HPLC chromatogram of JS-118 in the studied samples. **a** Cabbage blank, **b** Spiked cabbage at 0.01 mg/kg, **c** Spiked cabbage at 0.1 mg/kg, **d** Spiked cabbage at 0.5 mg/kg, **e** Soil blank, **f** Spiked soil at 0.01 mg/kg, **g** Spiked soil at 0.1 mg/kg, **h** Spiked soil at 0.5 mg/kg)

230 nm, calibration range was liner from 0.05 to 10 μg/mL. The standard curve equation was $y = 38{,}080\,x + 690.7$ ($R^2 = 1$). Linearity correlation was shown in Fig. 2.

The mean recoveries of the pesticide (n = 3) at spiking levels (0.01, 0.1 and 0.5 mg/kg) appears from Table 1. Satisfactory results were found in the three instances, with

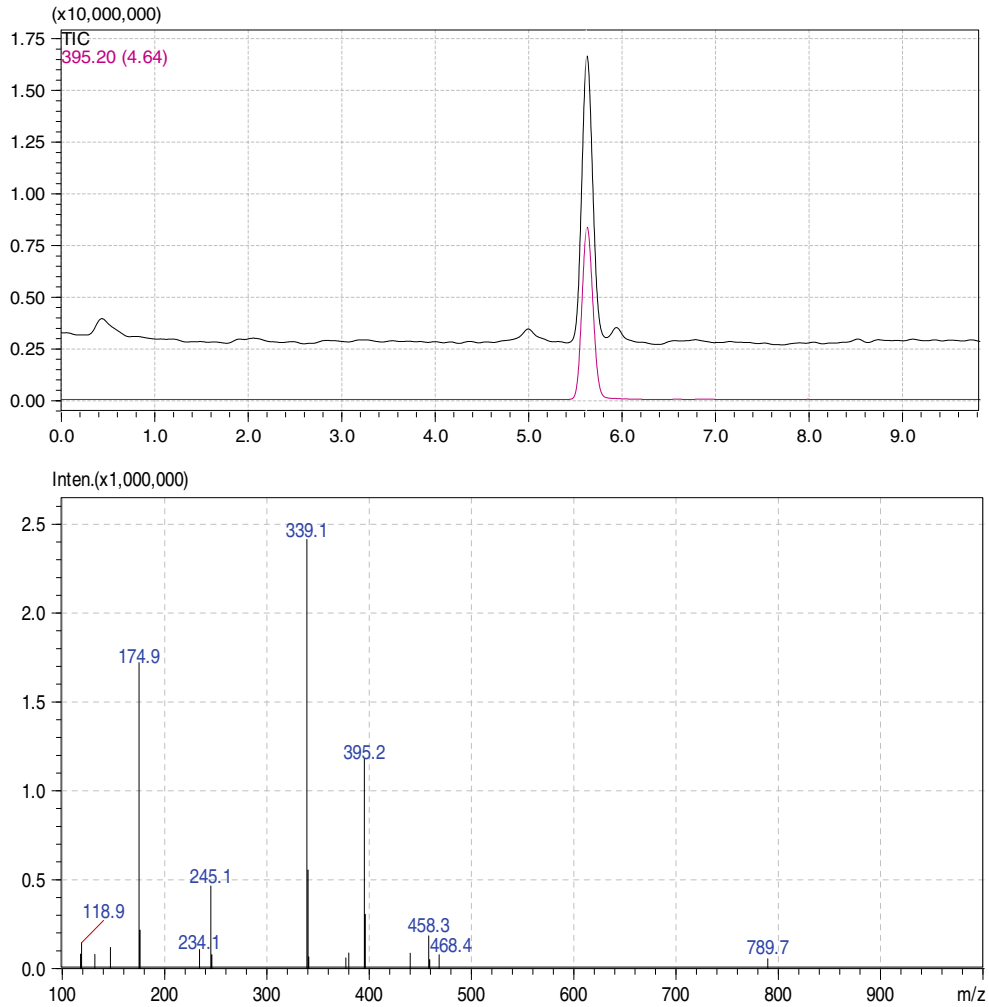

Fig. 4 Chromatogram of TIC and LC-MS

recoveries between 96.6% and 107.0%. Figure 3 shows the chromatograms of the JS-118 blank and different levels of the spike.

The coefficient variation of the methods (CV %) for repeatability was ranging from 0.4% to 4.7%. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as the sample concentration of JS-118 at peak heights of 3 and 10 times the baseline noise, respectively. The limit of detection (LOD) was estimated to be 0.004 mg/kg the pesticide. LOQ of JS-118was found to be 0.01 mg/kg.

We have investigated PSA for cabbage and soil samples purification in this study. The pesticide was not retained by PSA when eluted with *n*-hexane/dichloromethane (6:4, v/v) while many interfering matrixes were adsorbed onto the solid phase. It indicated that PSA provides sufficient cleanup for the crude extracts of cabbage and soil, and our previous works have also confirmed the excellent cleanup efficiency of PSA for plant material extract (Hu and Yan 2008; Li and Hu 2006).

Confirmation tests by HPLC-MS were used to determine whether or not peaks detected at the retention times of the analyte were in fact JS-118. JS-118 was identified by the specific molecule ion peak ($[M + H]^+$ at m/z 395.2 in HPLC-MS according to the proposed conditions (Fig. 4).

In this paper, a rapid and simple HPLC-DAD method was developed and validated for determination of JS-118 residues in cabbage and soil. The method developed shows satisfactory validation parameters in terms of linearity, lower limits, accuracy and precision. The average recoveries in all matrices for the pesticide ranged between 96.6% and 107.0%. The uncertainty associated to the analytical method, expressed as CV, was lower than 4.7 % for the pesticide tested in all matrices. LOQ of JS-118 was found to be 0.01 mg/kg [the maximum residue limits (MRLs) have not been established by China or other country's legislations], and sensitive enough in residue analysis by HPLC. This proposed analytical procedure is fast, easy to perform and could be utilized for regular monitoring of JS-118 residues in vegetables, soil and natural water.

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