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RESEARCH PAPER

Characterization of Activation Metabolism Activity of Indoxacarb in Insects by Liquid Chromatography-Triple Quadrupole Mass Spectrometry

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Abstract: A triple-quadrupole LC-MS/MS method was developed for the investigation of indoxacarb *N*-decarbomethoxyllation activity catalyzed by insect crude enzymes. The metabolite, *N*-decarbomethoxyllated indoxacarb (DCJW), was determined using electrospray ionization in positive ion mode. The quantitative and qualitative analyses were performed in multiple reaction monitoring (MRM) mode. The relative standard deviation (RSD, n = 5) of DCJW was 2.1%, indicating the metabolite had a good chemical stability in 30 h after the sample preparation. Five injections of DCJW standard solution in acetonitrile, five injections of the same sample in one incubation (substrates plus enzyme samples), and five injections of 5 separate metabolic reactions with the whole body of a susceptible strain of *Plutella xylostella* (L.) were individually injected into LC-MS/MS system. The RSD was 0.7%, 1.1% and 2.7%, respectively. The average recoveries for 440, 880 and 2200 pg of DCJW added to incubation mixtures were 96.1%–102.9%, and the RSD of recoveries of three added levels ranged from 4.8% to 9.4%. The limits of quantification and detection were 0.1 and 0.01 pg, respectively. A good linearity was achieved in the range of 46–2310 pg (R^2 = 0.9996). This method with high sensitivity and simplicity was applicable to the assay of indoxacarb *N*-decarbomethoxyllation activity of insect metabolic enzymes. A comparison of indoxacarb *N*-decarbomethoxyllation activity of an avermectin-resistant strain and a susceptible strain of *Plutella xylostella* (L.) was carried out using this novel LC-MS/MS method. The results showed that the indoxacarb *N*-decarbomethoxyllation activity in the resistant strain was 3.4-fold of that in the susceptible strain, implicating that negative cross-resistance might exist between indoxacarb and avermectin in *Plutella xylostella* (L.).

Key Words: Liquid chromatography-triple quadrupole mass spectrometry; Indoxacarb; Activation metabolism; *Plutella xylostella* (L.)

1 Introduction

The metabolic enzymes that mediate direct insecticide metabolism in insects are mainly comprised of cytochrome P450s[1], hydrolases[2], glutathione-S-transferases[3], etc. The metabolism of insecticides catalyzed by metabolic enzymes in insects has great toxicological significance, performing at detoxification or/and activation metabolism, or the balance of the two metabolisms. The activity of the insecticide metabolism affects the fortune in insects, action mechanism, biological activity, and resistance level of the insecticide[4]. In the study of entomology toxicology, especially for the resistance biochemical mechanism of insects resistant to insecticides, the classical spectrum, such as UV vis colorimetry and fluorometry, was commonly applied to the determination of the metabolism activity of insects to various model substrates or insecticide analogues[5]. Recently, the methods based on chromatography or chromatography/mass

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spectrometry have been described[6–9], however, few of them were used to characterize the direct metabolism activity of insects to insecticides[5]. As the determination of the metabolism activity of insects to insecticide itself is more conclusive than that to model substrates (one or more substrates) for the characterization of metabolism activity to insecticide [1], the development of an analytical method for the metabolism activity of insects to insecticides is clearly needed in the field of entomology toxicology.

Indoxacarb (DPX-JW062), a new oxadiazine insecticide developed by the DuPont Company, can efficiently control lepidopteran pests. The mechanism of action is that indoxacarb can be converted to the metabolite, *N*-decarbomethoxyllated indoxacarb (DCJW), in body fat and especially in the middle gut of lepidopteran insects. The formed DCJW can break the Na^+ channel in the nerve cells of pests, leading to the loss of function to death of pests[10,11]. To date, few works have been reported on the determination of the activation metabolism activity of indoxacarb in insects. In this study, an accurate analytical method was developed using high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) in multiple reaction monitoring (MRM) mode for the determination of the activation metabolism activity of indoxacarb in insects (Fig.1)[10,11]. The analytical parameters of MRM were optimized based the DCJW formed by the crude enzymes of the *H. armigera* larval midgut, and the novel assay method was ultimately established and validated using the DCJW produced by the crude enzymes of *Plutella xylostella* (L.). The quantitative and qualitative analyses of DCJW in MRM mode were performed at *m/z* 267.0 and *m/z* 207.0, 150.0, to characterize the hydrolysis activation metabolism activity. This method was applied to compare the activation metabolism activity between an avermectin-resistant strain and a susceptible strain of *Plutella xylostella* (L.) to insecticide indoxacarb.

2 Experimental

2.1 Instruments and reagents

An Agilent 1260/6460 high performance liquid chromatograph/triple quadrupole mass spectrometer (Agilent, USA) was equipped with a MassHunter Workstation Software (Version B.04.00, Agilent, USA). A T6-Newcentury UV-VIS Spectrometer was purchased from PGENERAL Instruments Co., Ltd., Beijing, China. A CR22E high-speed desktop centrifuge was from Hitachi Koki Co., Japan.

Indoxacarb (99.0% purity) was kindly supplied by the Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), Beijing, China. DCJW (99.0% purity) was supplied by Shenyang Research Institute of Chemical Industry, China. Formic acid (98.0% purity) was from Sigma-Aldrich Co., USA. HPLC-grade acetonitrile and acetone were purchased from Fisher Co., USA. Water was obtained using a Milli-Q water purification system. Bovine serum albumin (BSA) was from Beijing Tongzheng Biology Co., China. Analytical grade of ammonium acetate was purchased from Beijing Chemical Reagents Co., China.

2.2 Chromatographic conditions

The chromatographic separation was carried out using a ZORBAX Eclipse XDB-C_{18} (100 mm × 2.1 mm, 3.5 μm, Agilent, USA) equipped with a pre-column (10 mm × 2.1 mm, 5 μm, the stationary phase was the same to the analytical column). The mobile phase in gradient mode consisted of acetonitrile and water (0.1% HCOOH). The gradient of acetonitrile was set at: 15%–80% in 0–15 min; 80%–95% in 15–25 min; 95%–100% in 25–26 min; 100% in 26–29 min; 100%–15% in 29–31 min; 15% in 31–37.5 min. The flow rate was set at 0.4 mL min^{-1}, and the column temperature was set at 30 ºC. The injection volume was 15 μL.

2.3 MS/MS conditions

For the MS/MS analysis, ESI was performed in positive ionization mode. The mass scan ranged from *m/z* 100 to 800, and the fragmentor voltage was 100 V. The capillary voltage and atomizing gas pressure were 4.0 kV and 275 kPa, respectively. The flow rate of drying gas was 10 mL min^{-1} and the temperature of solvent removal was 350 ºC. Nitrogen was used as collision gas. The triple quadrupole mass spectrometer was operated in MRM mode with *m/z* 470.1/267.0 (collision energy of 10 V, dwell time of 300 ms) as the quantitative ion and *m/z* 470.1/207.0, 150.0 (collision energy of 30 V and dwell time of 100 ms for each) as the qualitative ions.

2.4 The tested insects

Cotton bollworm population, *Helicoverpa armigera* (Hübner),

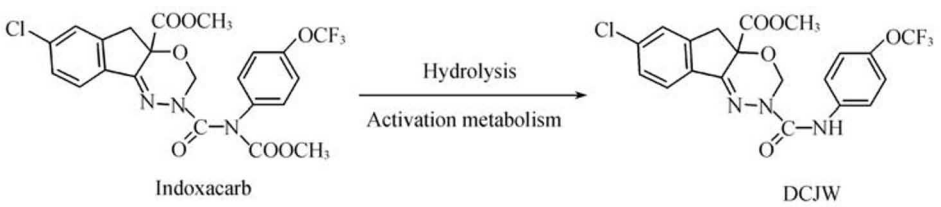

Fig.1 Bioactivation of indoxacarb in insects to *N*-decarbomethoxyllated metabolite (DCJW) by hydrolysis