RAPID ASSAYS BASED ON MONOCLONAL ANTIBODIES FOR PYRETHROID INSECTICIDE RESIDUES IN RAPESEEDS

Qi Zhang[1,2], Peiwu Li[1,2,3,*]
[1] Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan, 430062, P.R. China
[2] Key Laboratory of Oil Crop Biology of the Ministry of Agriculture, Wuhan, 430062, P.R. China
[3] Quality Inspection and Test Center for Oilseeds Products of the Ministry of Agriculture, Wuhan, 430062, P.R. China
* to whom correspondence should be addressed at: Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan, 430062, China
Tel: +86-27-86812943; Fax: +86-27-86812862; Email: peiwuli@oilcrops.cn

Abstract

(Background) Rapeseeds with low or without synthesized chemicals such as pyrethroid residues are very important not only for cold pressed rapeseed oil quality but also for shoots used as vegetable. Whereas, pyrethroid insecticides, such as esfenverate and deltamethrin, were used widely to protect rapes from pests, and their residues have great chance to become new contaminants in original oil owing to cold processing without refining step, which remains rapeseed components, including contaminants, in products as many as possible. So a rapid assay for monitoring raw rapeseed material is essential to cold pressed oil.

(Objectives) we aimed to develop some new rapid immunoassays for monitoring rapeseed contaminants such as fenverate and deltamethrin.

(Methods) To develop rapid assays for pyrethroids in rapeseeds, six monoclonal antibodies (mAbs), with IC_{50} (target concentration of 50% inhibition) range of 17.0-94.2 ng mL^{-1}, were obtained against immunogens of deltamethrin (hapten II-BSA) or esfenvalerate (compound 7-BSA). Among them, monoclonal antibody (mAb) 5B10 against esfenvalerate and mAb 2B12 against deltamethrin were selected for assay development owing to their higher specificity and sensitivity than others. With mAb 5B10 and 2B12, an immunoaffinity column, which can specifically capture both of esfenvalerate and deltamethrin, was prepared for sample cleanup. Also, enzyme-linked immunosorbent assays (ELISA) and lateral flow strip assay were developed.

(Results) Of two optimized ELISA, dynamic range was 16 - 13316.5 ng mL^{-1} for esfenvalerate and 0.5-499.8 ng mL^{-1} for deltamethrin. For validation, rapeseeds, spiked with standards of esfenvalerate or deltamethrin, were analyzed with the ELISAs and an authorized GC method. About 80% of recovery of the ELISAs was observed, which was little lower than the authorized method. The results indicate that the developed ELISAs can meet the requirement of pyrethroid residues analysis in rapeseeds. Additionally, a nanogold immunochromatographic assay for esfenvalerate was developed with a competitive format. The lowest concentration of target analytes was 800 ng mL^{-1} when no any red color on test line (inhibited completely), which inferred that the visual sensitivity was very high.

(Conclusion) The Rapid immunoassays based on mAbs provide a suitable method for screening agro-products including rapeseeds contaminated by esfenverate and deltamethrin.

Keywords: rapeseeds, immunoassay, monoclonal antibody, pyrethroid, insecticide residue

1. Introduction

Rapeseed oil is one of the most important vegetable oils in the world including (Koski et al., 2002), and China is not an exceptional. Generally, there were about 12 million tons of rapeseeds harvested in China every year, and close to 60 million tons of them totally all over the global. In order to ensure rapeseed production, pyrethroid insecticides, such as esfenverate and deltamethrin, were used widely to protect cruciferae from pests (Murchie et al., 1997; Slater et al., 2011). On the other hand, rapeseeds cold-pressed oil had been proved to be favourable effects on circulating LDL cholesterol and oxidized LDL, which may be important in the management of patients at high cardiovascular risk (Palomäki et al., 2010). And with development of cold pressing, original cold pressed rapeseed oil has being preferred by more and more consumer (Matthaus and Bruhl, 2003). It is reasonable that pyrethroid insecticide residues have great chance to become new contaminants in original oil owing to cold processing without refining step, which remains rapeseed components, including contaminants, in products as many as possible, and rapeseeds with low or without synthesized chemicals such as pyrethroid residues are very important for cold pressed rapeseed oil quality. So a rapid assay for monitoring raw rapeseed material is essential not only to vegetable shoots but also to cold pressed oil.

Immunoassays for chemicals in agro-products have been considered as a valuable supplement to existing, and rapidly developing, chromatographic techniques, owing to their attractive features including high sensitivity and selectivity, rapid detection (including low cost, time-saving, high throughput and on-site monitoring use), and the possibility of analysis of difficult matrices without extensive pre-treatment (Li et al., 2009; Zhang et al., 2010).

2. Materials and methods

2.1. Chemicals and instruments

The pyrethroid insecticide standards and chemicals for hapten synthesis were obtained from Jiangsu Pesticide Research Institute (Nanjing, China) or Chemphy Chemical Co., Ltd. (Shanghai, China). Biochemicals of Goat anti-mouse IgG-HRP, BSA, culture elements were purchased from Sigma-Aldrich (USA). The ELISA was carried out in 96-well polystyrene microplates (Costar, USA) and red by a SpectraMax M2e microplate reader (Molecular Devices, USA). For validation, a standard method of gas chromatography (Agilent 7890, Agilent Technologies Inc.) was used to detect the spiked samples.

2.2. Preparation of manual antigen and monoclonal antibodies (mAbs)

The manual antigens of deltamethrin were synthesized by the schemes in figure 1a, and those of esfenvalerate in figure 1b. With hapten II-BSA as immunogen, mAb 2B12 was gotten, and with compound 7-BSA to obtain mAb 5B10 (Jiang et al., 2010; Kong et al, 2010). Some other mAbs, such as mAb 4E3 and so on, were obtained later. Totally, six mAbs, with IC_{50} (target concentration of 50% inhibition) range of 17.0-94.2 ng mL^{-1} were obtained.

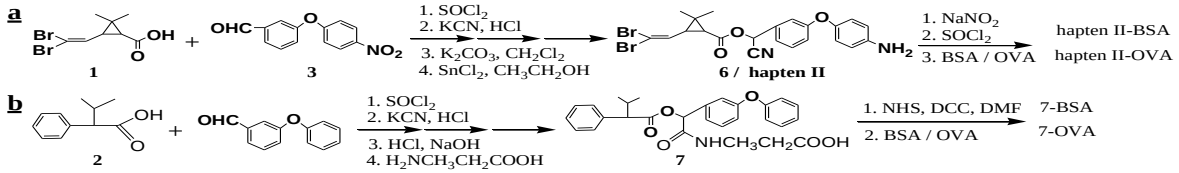

Figure 1 Synthetic schemes for the manual antigens of deltamethrin and esfenvalerate

2.3. Preparation of immunoaffinity columns and lateral flow immunostrips

The above mAbs was conjugated with amino group silica gel micro-particles by the classic EDC method, and then the conjugates was dispatched into 1 ml polyacrylamide syringe to prepare immunoaffinity columns for pretreatment of sample containing pyrethroids.

The antibodies were also conjugated with nano-gold particles to prepare immunostrips for lateral flow assay according to our reported procedure (Zhang et al., 2011).

2.4. Sample analysis by immunoassays and GC method

For validation of the developed immunoassays, several samples, such as rapeseed, rapeshoots, cabbage, green pepper, tea, environmental water and so on, was spiked with pyrethroid standards to a range of 10-1000 ng g^{-1} (or ng mL^{-1}). And then these spiked samples were detected by the developed immunoassays and an authorized GC method, in which the analyte was separated by quartz capillary column at a programmed temperature (150°C for 2 min, 6°C per min, 270°C for 23 min) and was determined by electron capture detector at 320°C.

3. Results

3.1. Characterization of monoclonal antibodies

Specificity of mAb 2B12 and 5B10 were tested and both of them were shown little or no cross-reactivity (0.0-25.9%) with other tested pyrethroids and their metabolites.

3.2. Optimization of competitive immunoassays

Several factors with great potential effects on assay sensitivity were optimized, and the final parameters for standard immunoassay curves were shown in table 1.

Table 1 The optimized items and parameters for development of immunoassays

item	the final selected for immunoassay curve	
	deltamethrin	esfenvalerate
coating hapten and its concentration	hapten II,	hapten IV, 2 μg mL^{-1}
antibody and ascites dilution	2B12, 1: 250000	5B10, 1: 32000
organic co-solvent	20% methanol	10% methanol
blocking reagent	1% OVA	1% OVA
IC_{50}	17.0 ± 3.3 ng mL^{-1}	94.2 ng mL^{-1}
assay range (from IC_{80} to IC_{20})	0.501 - 499.8 ng mL^{-1}	16 - 13316.5 ng mL^{-1}

3.3. Validation of the developed assays

The spiked samples containing deltamethrin or esfenvalerate were tested by the developed immunoassays and an authorized GC method at almost same time. And recoveries by the ELISAs were ranged from 72% to 108% for deltamethrin and from 76% to 109% for esfenvalerate, averaged about 80%, little lower than the authorized method (table 2). The results of immunostrip assays (positive or negative) were all accordance with the actual.

Table 2 Validation results of the developed immunoassays by authorized GC method

sample	spiked ng g^{-1}/ng mL^{-1}	analyte	recovery by		
			immunostrip	ELISA	GC
rapeseed, rape, cabbage, etc	10, 100, 150, 1000	deltamethrin	no detection	average 82.3% (72% - 108%)	average 96.4%
		esfenvalerate	accordance	average 80.6% 76% - 109%	average 93.2%

4. Conclusion

The Rapid assays developed here based on specific monoclonal antibodies 2B12 and can be suitable well for screening agro-products including rapeseeds contaminated by esfenverate and deltamethrin.

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