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Determination of Methoprene in Grains by Immunoassay

Byungchul Kim[1], Titan Fan[1] and Richard Moorman[2]

[1]Beacon Analytical Systems, Inc., Saco, ME, USA

[2]Wellmark International, Dallas, TX, USA

Abstract

Methoprene is an insect growth regulator used to control mosquitoes, beetles, fleas and moths. A competitive enzyme-linked immunosorbent assay (ELISA) for detection of methoprene was developed. Rabbit polyclonal antibodies were produced after immunization and used for the assay development. Total assay time was less than 70 minutes. The detection limit for methoprene in 10% methanol/PBS buffer was 10 ppb. Methoprene on grain samples (barely, corn, oat, rice and wheat) was extracted with acetonitrile with shaking. The extract was filtered by a packed glass wool, and the filtrate was diluted with 10% methanol/PBS. The detection range for methoprene in grain sample was 1 ppm to 25 ppm. The recoveries of spiked methoprene (5 and 10 ppm) were determined by ELISA and HPLC. The ELISA method gave slightly over recoveries ranging from 100 to 133%. The recovery determined by HPLC method ranged from 83.0 to 93.5%. Grain samples purchased from a different market were used for recovery test to see whether the good recovery is reproducible, and the recovery determined by ELISA ranged from 79.6% to 125%. The results indicate that the ELISA kit developed in this study (96-well plate format) can be used as a rapid and accurate tool for the determination of methoprene in various grain samples.

Introduction

Methoprene is an insect growth regulator used to control insects such as mosquitoes, flies, moths, and fleas. Methoprene interferes with normal maturation process of insects that eventually prevents them from reproduction. Methoprene was first registered as a conventional, chemical pesticide by US EPA in 1975 and later re-classified by the agency as a biochemical pesticide. Commercial methoprene products are available with different trade names including Altosid®, Precor®, Kabat®, Pharoid®, Dianex®, Apex®, Fleatrol®, Ovtrol®, Extingush® Diacon®, Minex® and Z-515®. Methoprene is used in the production of many foods including meat, milk, mushrooms, peanuts, rice and cereals. It is also used for domestic pets to control fleas. Based on US FDA regulation, methoprene is exempt from the requirement of tolerance in or on all food commodities when used to control insect larvae. The maximum treatment rate for stored grains is 5 ppm and no health hazards have been reported that could be related to consumption of methoprene treated grains. It also has been known that risk from consumption of treated food commodities is considered negligible for infants, children and adults (EPA, 2001). However, CODEX Alimentarius indicates maximum residue limits for methoprene in various commodities (Table 1). Various analytical methods have been developed such as HPLC-UV, GC-MS and LC-MS/MS (Abu-Qare and Abou-Bonia, 2001). However, those analytical methods need extensive and time consuming sample preparation procedures.

Therefore, development of simple and easy methods to detect methoprene has been demanded. Enzyme linked immunosorbent assay has been considered as a rapid method and developed by several research groups (Hill et al, 1991 and Wang et al, 2002). In this study, a competitive ELISA was developed and evaluated to determine methoprene in various grain samples.

Table 1. Maximum Residue Limits for Methoprene 2006 (CODEX Alimentarius).

Commodity	MRL (mg/kg, ppm)
Cereal grains	10
Edible offal (mammalian)	0.02
Eggs	0.02
Maize oil, Crude	200
Meat (from mammals other than marine mammals)	0.2
Milks	0.1
Poultry meat	0.02
Poultry, Edible offal of	0.02
Rice hulls	40
Wheat bran, Unprocessed	25

Methodology

Polyclonal antibody production:

In the development of this assay, methoprene was coupled to a protein carrier, which served as an immunogen, and horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. The serum was collected monthly.

Sample preparation:

1. Weigh 5 g of sample (whole grain) into a glass vial.
2. Add 5 mL of acetonitrile.
3. Shake vigorously by hand for 30 seconds.
4. Let it sit overnight in a dark place.
5. Filter the liquid portion with a syringe filter (0.2 µm).
6. Dilute the filtrate with sample diluent 100 times. (0.1 mL/10 mL)
7. Run the assay

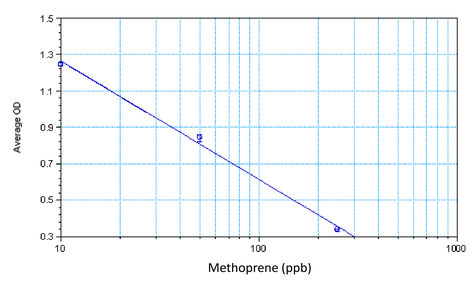
HPLC Condition

- Column: ZORBAX Eclipse XDB-C18 (150 X 4.6 mm, 5 µm)
- Mobile phase: Methanol/Water (85/15)
- Flow rate: 1 mL/min
- Detector: UV 265 nm

Assay protocol:

1. Add 50 µl of HRP conjugate to each well.
2. Add 50 µl of calibrator or sample extract.
3. Add 50 µl of antibody solution to the wells and mixed gently for 30 seconds
4. Incubate the wells for 30 minutes.
5. Dump the well contents and wash 4 times with PBST wash solution.
6. Add 100 µl of substrate solution into each well and incubate for 30 minutes.
7. Add 100 µl of stop solution and read at 450nm-650nm.
8. The concentration is calculated from a calibration curve applying dilution factor (100).

Results



Calibration curve (R^2 = 0.997)

Table 2. OD values of calibrators.

Well Contents	OD	Average OD ± SD	%RSD	%Bo
Negative Control	1.417 1.411	1.414 ± 0.004	0.3	100
10 ppb Calibrator	1.213 1.223	1.218 ± 0.007	0.6	86
50 ppb Calibrator	0.815 0.799	0.807 ± 0.011	1.4	57
250 ppb Calibrator	0.310 0.323	0.316 ± 0.009	3.0	22

Table 3. Comparison of ELISA and HPLC for the determination of Methoprene.

Grain	Spike (ppm)	ELISA		HPLC	
		Methoprene (ppm)	Recovery (%)	Methoprene (ppm)	Recovery (%)
Corn	5.00	5.01	100	4.15	83.0
	10.0	11.9	119	8.75	87.5
Oat	5.00	5.92	118	4.55	91.0
	10.0	13.3	133	9.10	91.0
Rice	5.00	5.49	110	4.61	92.2
	10.0	12.4	124	9.35	93.5
Wheat	5.00	5.04	101	4.44	88.8
	10.0	11.1	111	9.12	91.2

Results

Table 4. Recovery of methoprene spiked in grain samples obtained from a different market.

Grain	Spike (ppm)	ELISA	
		Methoprene (ppm)	Recovery (%)
Barley	5.00	4.45	89.0
	10.0	9.25	92.5
Corn	5.00	3.98	79.6
	10.0	8.05	80.5
Oat	5.00	4.25	85.0
	10.0	9.23	92.3
Rice	5.00	5.42	108
	10.0	12.3	123
Wheat	5.00	5.47	109
	10.0	12.5	125

Discussion

A competitive immunoassay was developed for determination of methoprene in grains. Methoprene was extracted from whole grain(s) with acetonitrile overnight. Shorter extraction times (5 hours or less) or vortexing (3 minutes) resulted in poor recoveries (Data not shown). Due to instability of methoprene in aqueous solution, concentrated methoprene (25 ppm) in organic solvent was prepared, and fresh calibrators were made each time by dilution (100 times) of the concentrated methoprene. When stored at 37 °C, the concentrated methoprene (25 ppm/Acetonitrile) and methoprene-HRP conjugate were stable at least 5 months and 6 months, respectively. It was found that the use of plastic bottle (or vial) should be avoided due to potential attachment of methoprene on the plastic surface. Therefore, glass vial or bottle for sample extraction and calibrator dilution is preferred. Four different grains were spiked with known concentrations of methoprene, and tested with the developed immunoassay and HPLC. The immunoassay gave slightly over recoveries ranging from 100 to 133%. The recovery determined by HPLC ranged from 83.0 to 93.5%. Grain samples purchased from local markets were spiked with two concentrations of methoprene and tested. The recovery from this second sample set ranged from 79.6% to 125% showing similar recovery range with the first test. These results indicate that the ELISA kit developed in this study (96-well plate format) can be used as a rapid and accurate tool for the determination of methoprene in various grain samples.

References

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