Bull Environ Contam Toxicol (2012) 88:555–558
DOI 10.1007/s00128-012-0541-y

Extraction, Cleanup, and Chromatographic Determination of Imidacloprid Residues in Wheat

Sajid Iqbal · Riaz Uddin · Sumayya Saied · Mubarik Ahmed · Muhammad Abbas · Sahar Aman

Received: 28 September 2011 / Accepted: 25 January 2012 / Published online: 7 February 2012

Abstract This study presents an improved method for quantitative analysis of imidacloprid residues in wheat grain using high performance liquid chromatography. The study used chromatographic response (in terms of peak height) as a quantitative tool for determination instead of peak area. The peak height of imidacloprid showed a very good linear correlation ($R^2 = 0.999$) when compared with absolute values at six different concentrations. The limit of detection was found to be 0.01 μg/mL. The recovery of imidacloprid residues in spiked wheat grain at three levels (0.03, 0.05, and 0.1 μg/g) was in the range of 79%–88% with %RSD 5.72 at 0.05 μg/g (w/w) and between 87% and 93% with %RSD 3.55 at 0.1 μg/g (w/w). At 0.03 μg/g (w/w) level, recovery was not within the recommended range of 70%–110%. Therefore, the lowest limit of quantification for this method was found to be 0.05 μg/g.

Keywords Imidacloprid · Wheat grain · HPLC

Wheat is the staple food grain in many part of the world. It is one of the cheapest sources of nutrition and protein in much of the developing world. Wheat production is also prone to losses due to insect pests (Masud and Parveen 1991). According to some estimates, pest attack may reduce the production of wheat by 46% to 83% (Saltmarsh 2005). In many part of the world, imidacloprid is routinely used to protect wheat from insect pest attack. Imidacloprid is applied in multiple ways, e.g. in the form of seed treatment, soil drench, or foliar spray (Mohapatra et al. 2011). The mode of action of imidacloprid makes it different from many of the other insecticides (Joshi et al. 2009), and is considered an effective systemic insecticide. On application, it translocates to various plant parts, and exerts insecticidal action through effects on insect nervous system which interferes with transmission of stimuli and hence loss of activity and coordination (Bonmatin et al. 2003). The application of any insecticide to food crop draws concerns over food safety and requires a careful monitoring of the residues. In parts of the world, where imidacloprid is used without due regard to dosing and applications regimes and operators are not adequately trained, there is a likelihood that the residues may exceed the threshold limits. The study of residues of imidacloprid in wheat grain is therefore important from food safety point of view. High performance liquid chromatography (HPLC) technique is highly relevant for quantitative determination of imidacloprid residues. Nobuko et al. (2003) reported determination of imidacloprid in different agricultural products using HPLC. Similarly, a quantitative analysis of imidacloprid in wheat-seed and soil has been reported (Guiying et al. 2006). Similarly, Samnani et al. (2011) have reported the quantitative determination of imidacloprid residues in soil and water samples.

The current study was carried out for developing a method for quantitative analysis of imidacloprid in wheat grain using HPLC.

Materials and Methods

Control wheat samples (free from imidacloprid) were obtained from crop disease research institute, PARC,

S. Iqbal · R. Uddin (✉) · M. Ahmed · M. Abbas · S. Aman
Grain Quality Testing Laboratory, Southern zone Agricultural Research Centre, Pakistan Agricultural Research Council, Karachi University Campus, Karachi 75270, Pakistan
e-mail: riaz_1969@yahoo.com

S. Saied
Department of Chemistry, University of Karachi, Karachi 75270, Pakistan

Karachi. All solvents (i.e. acetone, methanol, acetonitrile, and dichloromethane) and other chemical reagents (i.e. anhydrous sodium sulphate and graphitized charcoal), purchased from Merck (Germany), were of analytical grade. Acidic aluminum oxide pH (4.0–5.0) of Brockmann activity 1 was procured from Fluka (Switzerland). The standard of imidacloprid (analytical grade, purity 98.80%) was purchased from accustandard, Inc (USA). Aluminum oxide and charcoal were activated at 500°C for 3 h and at 300°C for 3 h respectively. Anhydrous sodium sulphate was dried at 500°C for 4 h. Chromatography glass column of 58 cm length and 2.4 cm id, filter papers (Whatman # 542), and autoclavable micropipette (20 µL–200 µL)—Nichipet Ex Plus were used. Some of the supporting laboratory techniques, such as Bandelin Sonorex ultrasonic device, a rotary evaporator Model BUCHI V-512 with chiller, centrifuge machine Jouan-Inc (USA) Model # CR412 were also used.

Four grams of control wheat grain free from imidacloprid were spiked separately at three separate doses i.e. 0.01 µg, 0.05 µg and 0.1 µg of imidacloprid per gram sample weight. The spiked samples were kept for 24 h before extraction, for allowing time for insecticide penetration into the samples. The extraction and cleanup were undertaken as method described by Uddin et al. (2011). For this, 4 g of ground wheat was placed in 50 mL centrifuging tube and shaken vigorously after addition of 40 mL mixture of acetone:methanol (1:1). The samples were centrifuged at 2,500 rpm for 3 min and supernatant was passed through Whatman filter papers and collected in a separating funnel. The extraction was repeated by addition of another 35 mL of solvent mixture and centrifuging step. The two extracts were combined (approx 75 mL). Dichloromethane (25 mL) was added in the flask followed by 200 mL of 2.5% (w/v) sodium sulfate to remove any traces of moisture. After vigorous shaking, dichloromethane (DCM) was allowed to settle together with the analyte, and collected into the glass column containing 25 g of anhydrous sodium sulfate. Glass wool was used to stop sodium sulfate passing into the separating solvent. This process was repeated twice by adding 50 mL of DCM (in two equal portions). The residual sodium sulfate was washed with another 10 mL DCM. The collected moisture-free extract was concentrated to 1 mL–2 mL by rotary evaporation. The extract was then transferred to a glass column—filled with 13 g (12:1) of homogenized mixture of acidic aluminum and activated charcoal between layers of sodium sulfate. The extract was eluted through the column using 160 mL of DCM and the elutent was concentrated to dryness on a rotary evaporator. Finally, the residues were dissolved in 2 mL acetonitrile for further analysis.

All the samples were filtered through Millipore filter paper (pore size 0.45 µm) before they were injected in SHIMADZU HPLC equipped with UV–VIS Detector SPD-10AV and two high pressure pumps LC-10AT. Beckman C18 column (5 µm, 4.6 mm × 15 cm) was used as the stationary phase. Acetonitrile and water, placed in separate reservoir linked with two separate pumps, were used as mobile phase. The pumps were programmed to mix and pump the mobile phase at a ratio of 8:2 of acetonitrile: water, at flow rate of 0.7 mL/min. Total run time for each analysis was programmed for 10 min, mobile phase was allowed to flow for 5 min at 1.0 mL/min flow rate after each run to remove any traces of the analyte that may cause artifacts in the next analysis.

Results and Discussion

In the present study, extraction and cleanup steps were performed according to Uddin et al. 2011, with some modifications in the cleanup step. Dry mixture of acidic alumina and activated charcoal was introduced into glass column with the help of aluminum foil instead of slurry of that mixture in dichloromethane to reduce the amount of DCM used in the cleanup step. For the quantitative analysis of imidacloprid residues, HPLC linked with a UV detector was used. HPLC has an advantage over GC in terms of mild conditions for the determination of analytes. During this study, different parameters were taken into consideration for the best performance of the method (i.e. different wavelengths and ratios of mobile-phase solvents). Among all the studied wavelengths; 270 nm was found to be the best for detection of imidacloprid in terms of either peak area or height (Fig. 1). In this study, height of the peak was considered more relevant in deriving results instead of peak area as the quantitative means due to interference of certain co-extractives in the sample extracts. With the increasing ratios of acetonitrile in the mobile phase, a continuous better height was obtained.

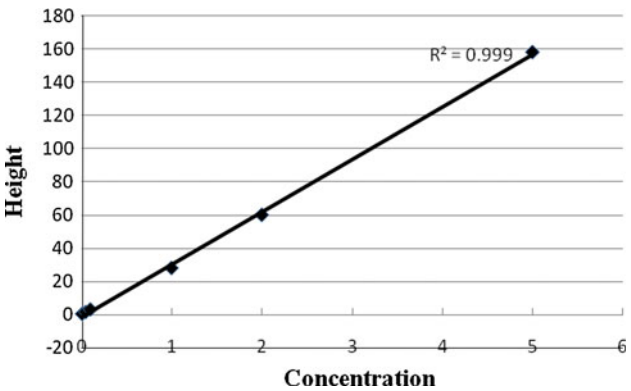

Fig. 1 Response of imidacloprid standard (2 µg/mL) at different wavelengths

Table 1 Optimization of mobile phase to identify best ratio of acetonitrile and water

ACN: water	Pressure (MPA)	Retention time	Area	Height	W05*
5:5	12.3	3.37	400.41	36.13	0.180
6:4	10.7	2.94	412.93	47.23	0.147
7:3	9.6	2.69	410.50	55.20	0.120
8:2	8.2	2.54	414.45	61.77	0.107
9:1	6.9	2.46	419.10	67.58	0.100
10:0	5.7	2.44	423.37	74.14	0.093

* Half width of the peak

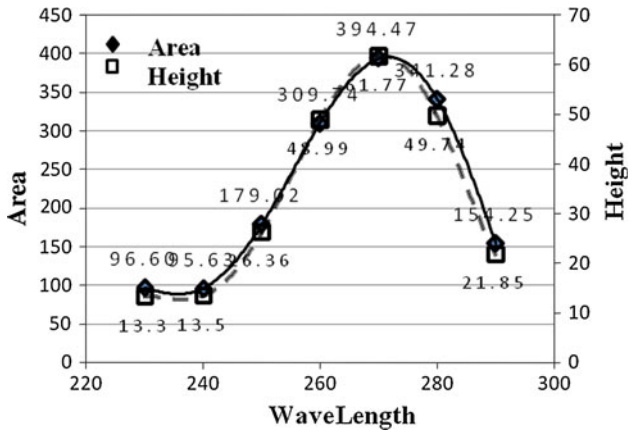

Fig. 2 Calibration between concentration (μg/mL) of imidacloprid and respective chromatographic response (peak height)

However, at 90% and 100% acetonitrile as mobile phase, imidacloprid peak overlapped the solvent-peak due to shifting of retention time. Therefore, optimum height producing mobile phase was found to be a mixture of 8:2 of acetonitrile: water) (Table 1). These results are in line with the findings of Ishii et al. (1994).

A number of method validation tests were used for determining the method performance. For this, six different dilutions ranging from 0.01 μg/mL to 5.00 μg/mL were prepared from a stock standard of imidacloprid. Plotting peak height of each of dilution against concentration showed a linear calibration with correlation coefficient of 0.999 (Fig. 2). This showed that peak height has a similar trend to that of relevant peak areas, and therefore either can be used for quantification purpose. Limit of detection (LoD) of imidacloprid was determined in relation to noise of the base line. As a result, LoD was found to be 0.01 μg/g where peak height remained around three times higher. Method performance was verified by spiking at three different levels (0.03 μg/g, 0.05 μg/g, and 0.1 μg/g). These samples were prepared by transferring calculated volume of the stock standard to each of the individual 4 gram control samples of ground wheat (free from imidacloprid residues). Accordingly, 0.05 μg/g was identified as the limit of quantification (LoQ) of this method. The recovery results are reported in Table 2. For 0.03, 0.05 and 0.10 μg/g spiked samples, recovery of between 61% and 72% with %RSD 8.66, 79%–88% with %RSD 5.72, and 87%–93% with %RSD 3.55. A widely accepted criterion for the acceptability of performance of an analytical method is its capability of providing average recovery within the range of 70%–110% (Hill et al. 2005). From the results obtained in this study, the recoveries of 0.10 μg/g and 0.05 μg/g are therefore within the acceptable range.

Table 2 Percent recoveries and percent RSD at different fortification levels

Fortification levels (in μg/g)	Percent recoveries			Average recovery	%RSD
	I	II	III		
0.03	72	61	64	65.67	8.66
0.05	88	79	81	82.67	5.72
0.10	92	87	93	91.00	3.55

In summary, this study has led to the development of a relatively straightforward and reliable method for determination of imidacloprid residues in wheat grain.

Acknowledgments Financial support of Pakistan Agricultural Research Council for undertaking the study is gratefully acknowledged. The authors are also grateful to the chairman, Department of Chemistry, University of Karachi, for his guidance.

References

Bonmatin JM, Moineau I, Charvet R, Fleche C, Colin ME, Bengsch ER (2003) A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. Anal Chem 75:2027–2033

Guiying X, Jie Y, Dandan S, Guohui Y (2006) Determining imidacloprid residues in wheat seed and soil. Henan province. People Repub China Nongyao 45:475–476

Hill ARC, Startin JR, Fussell RJ (2005) Analytical methods and analytical performance: comprehensive analytical chemistry. In: Amadeo R, Fernandez-Alba (eds) Chromatographic-mass spectrometric food analysis for trace determination of pesticide residues, vol XLIII. Chap.1. Elsevier, New York, p 25

Ishii Y, Kobori I, Araki Y, Kurogochi S, Iwaya K, Kagabu S (1994) HPLC determined of the new insecticide imidacloprid and its behavior in rice and cucumber. J Agric Food Chem 42:2917–2921

Joshi NK, Sharma VK (2009) Efficacy of imidacloprid (confidor 200 SL) against aphids infesting wheat crop. J Cent Eur Agric 10:217–222

Masud SZ, Parveen Z (1991) Persistence of permethrin, primiphos methyl and chlorpyrifos methyl insecticides in wheat stored under simulated environmental conditions. Pakistan J Sci Ind Res 34:240–246

Mohapatra S, Deepa M, Jagadish GK (2011) Behavior of beta cyfluthrin and imidacloprid in/on mango (*Mangifera indica* L.). Bull Environ Contam Toxicol 87:202–207

Nobuko N, Hideyuki S, Tetsuya H, Shoji T (2003) Analysis of imidacloprid in agricultural products. Wakayama-ken Kankyo Eisei Kenkyu Senta Nenpo 49:35–38

Saltmarsh M (2005) Food Safety/Pesticides, available on Encyclopedia of human nutrition, 2nd edn, pp 323–329. http://www.sciencedirect.com/science/article/pii/B0122266943003197#s005

Samnani P, Vishwakarma K, Pandey SY (2011) Simple and Sensitive Method for Determination of Imidacloprid Residue in Soil and Water by HPLC. Bull Environ Contam Toxicol 86:554–558

Uddin R, Iqbal S, Khan MF, Parveen Z, Ahmed M, Abbas M (2011) Determination of pesticide residues in rice grain by solvent extraction, column cleanup, and gas chromatography-electron capture detection. Bull Environ Contam Toxicol 86:83–89