The Journal of Animal & Plant Sciences, 26(1): 2016, Page: 170-176
ISSN: 1018-7081

DETERMINATION OF IMIDACLOPRID RESIDUES IN RICE FROM VARIOUS DISTRICTS OF PUNJAB USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Rice is Pakistan's major export crop and injudicious use of several pesticides by rice growers is making it all difficult for exporters to get their shipments clear regarding pesticide residues. In continuation of systematic research for determination of pesticide residues, rice samples were collected from key growers of important rice growing areas of Punjab including Gujranwala, Sialkot, Hafizabad, Narowal, Sheikhupura, Mandi Bahaudin, Pasrur and Jhang. Imidacloprid is a systemic insecticide with seed, foliar and soil uses to control sucking pests. Newly proposed maximum residual limit (MRL) for imidacloprid in rice by European food safety authority is 1.5 µg g^{-1}. High performance liquid chromatography with photo diode array detector was employed for imidacloprid residues analysis. Method validation data showed a recovery percentage of 86±6.12%, 115±3.53%, 108±6.01%, 93±3.53% and 93±3.84% for fortification levels of 0.01, 0.05, 0.1, 0.5 and 1 µg g^{-1} rice sample, respectively. Residues analysis data showed that most of the randomly selected samples carried imidacloprid residues. Three samples from district Sialkot (1.63, 2.22 and 2.74 µg g^{-1}) while one sample each from Gujranwala (2.63 µg g^{-1}) and Narowal (1.88 µg g^{-1}) exceeded the MRL. Imidacloprid residues were recorded in the range of 0.47 to 2.63 µg g^{-1}, 0.95 to 2.74 µg g^{-1}, < 0.01 to 1.16 µg g^{-1}, 0.43 to 1.88 µg g^{-1}, 0.01 to 0.82 µg g^{-1}, 0.07 to 0.76 µg g^{-1}, <0.01 to 0.14 µg g^{-1} and <0.01 to 0.1 µg g^{-1} rice in samples from Gujranwala, Sialkot, Hafizabad, Narowal, Sheikhupura, Mandi Bahaudin, Pasrur and Jhang, respectively. The information would be beneficial for rice exporters. Moreover, extension workers and their field staff should translate to the rice growers the threat their produce would be facing in modern international markets.

Key words: Imidacloprid Residues**,** Rice**,** Punjab, Pakistan, HPLC.

INTRODUCTION

Pesticides are used in killing of insects, animals, plant and fungal pests in domestic locations, agricultural fields and institutional settings. Public interest regarding pesticide toxicity has increased during the last few years due to significant evidence of carcinogenic and mutagenic effects in experimental animals and especially the exposed humans. The populace is generally exposed to chemical pesticides through consumption of contaminated food (cereals, fruit and vegetables), treated directly or indirectly with pesticides or grown in pesticides polluted fields (Tahir *et al*., 2009). A larger part of human population may therefore be affected, ranging from agricultural workers, their families, people living near farms/orchards, to the general masses exposed through the residues in food (Nasr *et al*., 2014).

Health problems associated with pesticide pollution include both acute and chronic toxicity. The acute effects may result in diarrhea, lacrimation, urination, diaphoresis, salivation and excitation of central nervous system (CNS). While, the chronic exposures are reported to cause allergies, neurotoxic and behavioral effects, cancer, hypersensitivities, disruption of the immune system and reproductive disorders (Tahir *et al*., 2009). Pakistani farmers depend exclusively upon hundreds of chemical pesticides to cope with the serious pest problems arising due to intensive crop production and the climate. Often, post-harvest protection is also the motive behind extensive and injudicious use of pesticides resulting adverse effects on human and the environment health. Modern international marketing system demands intensive research for determination of pesticide residues in different agricultural products (Singh *et al*., 2008).

Imidacloprid {1-(6 chloronicotinyl)-2-(nitroimino) imidazolidine} is a systemic insecticide with soil, seed, and foliar uses for the control of sucking pests (Sanyal *et al*., 2006) rather it is the most widely used insecticide (~ 70 crops in more than 100 countries) throughout the world (Bonmatin *et al*., 2003). It is commonly used on rice, maize, fruit, vegetables, sugar beet, cotton and turfs (Hassanzadeh *et al*., 2012). Imidacloprid residues have already been reported in various agriculture crops (Ishii *et al*., 1994; Alsayeda *et al*., 2008; Gupta *et al*., 2008; Hassanzadeh *et al*., 2012).

For determination of pesticide residues in rice, being important export of Pakistan, samples were collected from various districts of the Punjab. The objectives included; to provide basic information on

pesticide residues in rice and to generate data bank for establishment of maximum residual limit (MRL) of different chemicals required for food safety and export purposes.

MATERIALS AND METHODS

Equipment: Analysis was carried out using a Waters HPLC 2695. The system was equipped with Waters 2996 photo diode array detector and computer with Empower software. The HPLC column used was KROMASIL 100 with C18 as stationary phase, 5 µm pore size and 250 x 4.6 mm dimension purchased from Teknokroma®.

Chromatographic conditions: The mobile phase composition used was acetonitrile: water (65: 35% v/v). The analysis was carried out in isocratic mode at a flow rate of 0.8 mL/min, with column effluent being monitored at 270 nm UV wavelength.

Samples collection: Samples were collected from rice growers of important rice producing areas of the Punjab including Gujranwala, Hafiz Abad, Sialkot, Narowal, Mandi Bahaudin, Sheikhupura, Pasrur and Jhang. All samples collected in dark glass and labeled bottles, were stored in laboratory at 4ºC until prepared for pesticide residue analysis.

Sample preparation: The QuEChERS method (Anastassiades *et al*., 2003) was used with some modifications. Rice samples were ground to a fine homogenized powder using a standard food processor and homogenizer. Weighed 10 g powdered rice in centrifuge tube, added 10 mL distilled water and 10 mL Acetonitrile (HPLC grade) into it, vortexed to mix thoroughly. Added 4 g of $MgSO_4$ and 1 g of NaCl, shaked vigorously by hand for 1 minute followed by centrifuge for 5 minutes at 4000 rpm. The extract was passed through activated solid phase extraction (SPE) cartridge, shaked the filtrate vigorously by hand for 1 minute followed by centrifuge again for 3 minutes at 3000 rpm. Finally, the extract was transferred into 2 mL vial for analysis at HPLC after filtration with disposable nylon membrane filter (0.45 micron).

Insecticide standard solutions: The insecticide standard stock solution (1000 µg mL^{-1}) was prepared in acetonitrile followed by an intermediate standard solution of concentration 100 µg mL^{-1} by dilution with acetonitrile. From this intermediate solution suitable concentrations of working standards (in triplicate) were prepared using acetonitrile and analyzed immediately for getting the calibration curve and a linear equation. For checking cross-contamination, blank samples with all glassware and reagents except the active ingredient were also periodically run.

Method validation: To ensure analysis credibility, method validation parameters were determined including linearity, accuracy, precision and limits of detection (LOD) and quantification (LOQ). Linearity was determined by plotting resultant peak areas against different known concentrations (0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 and 10 µg mL^{-1}) and so obtained linear equation and value of regression coefficient (R^2). The method accuracy and precision was determined by recovery tests, with samples spiked at concentration levels of 0.01, 0.05, 0.1, 0.5 and 1 µg g^{-1}. Recovery was calculated using the following formula; (Hladik and McWayne, 2012)

$$R\% = [(C_S-C_U)/C_A] \times 100$$

Where: C_S is the analyte concentration determined from the spiked sample; C_U is the analyte concentration determined from the unspiked sample; C_A is the analyte concentration added in the spiked sample. The limit of detection (LOD, µg mL^{-1}) and limit of quantification (LOQ, µg mL^{-1}) was determined being the lowest concentration with a response of 3 and 10 times the baseline noise, respectively.

Sample analysis: Samples were analyzed with same instrument and processing methods as set with pure standards and spiked samples. Imidacloprid residues in samples were identified by matching the retention time with external standard. The concentrations in samples were calculated with the help of calibration curve.

RESULTS

Linearity: Various known concentrations of imidacloprid (10, 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005 µg mL^{-1}) were prepared in acetonitrile through dilution of the intermediate stock solution (100 µg mL^{-1}). The standard solutions were injected and measured the peak area. A calibration curve was plotted using concentrations of the known standards versus peak area observed. The linear regression equation so obtained was $Y = 69269X + 9096.3$ with $R^2 = 0.9967$ (Fig. 1).

Limits of detection and quantification: The limit of detection at which the response was at least three times the baseline noise found to be 0.005 µg mL^{-1}. While, the quantification limit at which the analyte peak was repeatedly generated at about 10 times the baseline noise was determined as 0.01 µg mL^{-1}.

Accuracy and Precision: Method accuracy was evaluated by performing recovery studies. Recovery test was done by analyzing three replicates of samples fortified at 0.01, 0.05, 0.1, 0.5 and 1 µg g^{-1} rice levels. The mean recoveries of imidacloprid from the spiked samples were 86 ± 6.12%, 115 ± 3.53%, 108 ± 6.01%, 93 ± 3.53% and 93 ± 3.84%, while the relative standard deviations were 12.37%, 5.32%, 9.61%, 6.59% and 7.19% for spiking levels of 0.01, 0.05, 0.1, 0.5 and 1 µg

g^{-1} rice, respectively (Table I). Since, no compound was detected that interfered with the sample, values were, therefore, not corrected for blank runs. Recoveries were adequate being in the acceptable range of 70-120%. Chromatograms of rice samples spiked with various concentrations of imidacloprid are given in Figure 2.

Residue analysis: A total of 80 rice samples, 10 from each of Gujranwala, Sialkot, Hafizabad, Narowal, Sheikhupura, Mandi Bahaudin, Pasrur and Jhang were analyzed for the presence of imidacloprid residues and the respective residue range in samples from these districts was found to be 0.47 to 2.63 µg g^{-1}, 0.95 to 2.74 µg g^{-1}, < 0.01 to 1.16 µg g^{-1}, 0.43 to 1.88 µg g^{-1}, 0.01 to 0.82 µg g^{-1}, 0.07 to 0.76 µg g^{-1},<0.01 to 0.14 µg g^{-1} and <0.01 to 0.1 µg g^{-1} (Table II). Out of ten samples collected from key rice growers of each selected districts, the detectable imidacloprid residues were found in 7, 8, 6, 5, 5, 3, 5 and 4 samples from Gujranwala, Sialkot, Hafizabad, Narowal, Sheikhupura, Mandi Bahaudin, Pasrur and Jhang, respectively. In total, 43 samples out of 80 (54%) contained imidacloprid residues. For mean imidacloprid residue level, the districts may be arranged in descending order as Sialkot (1.5 µg g^{-1} rice) > Gujranwala (0.88 µg g^{-1} rice) > Narowal (0.83 µg g^{-1} rice) > Hafizabad (0.57 µg g^{-1} rice) > Mandi Bahaudin (0.48 µg g^{-1} rice) > Sheikhupura (0.31 µg g^{-1} rice) > Jhang (0.08 µg g^{-1} rice) > Pasrur (0.06 µg g^{-1} rice). Data in Table II also showing that imidacloprid residues in three samples from district Sialkot (1.63, 2.22 and 2.74 µg g^{-1} rice) while in one sample each from Districts Gujranwala (2.63 µg g^{-1} rice) and Narowal (1.88 µg g^{-1} rice) exceeded the newly proposed EU MRL of 1.5 µg g^{-1} (EFSA, 2010).

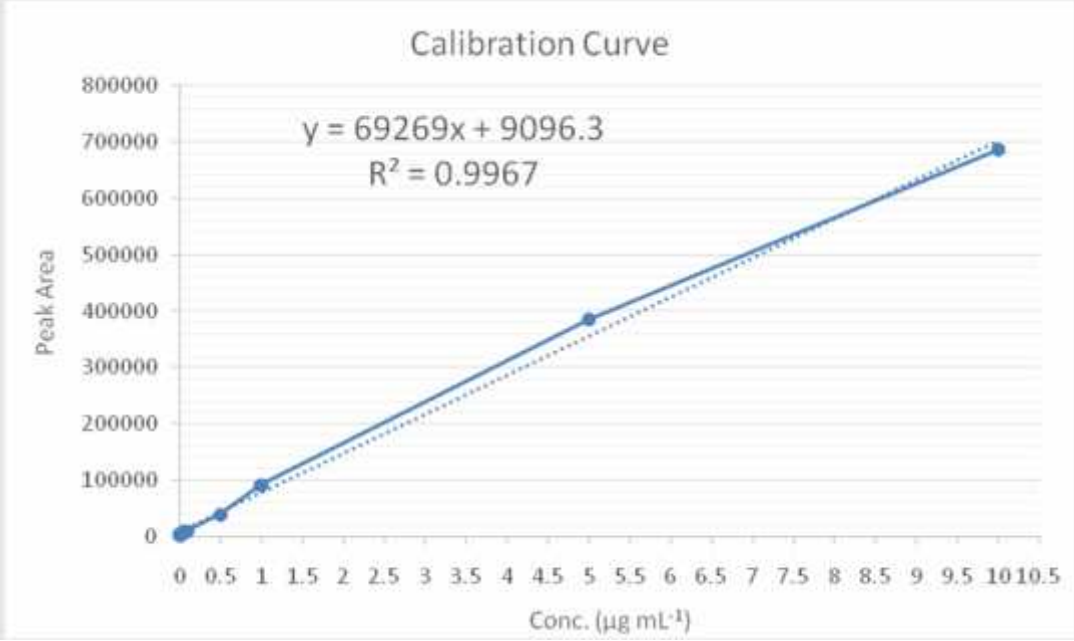
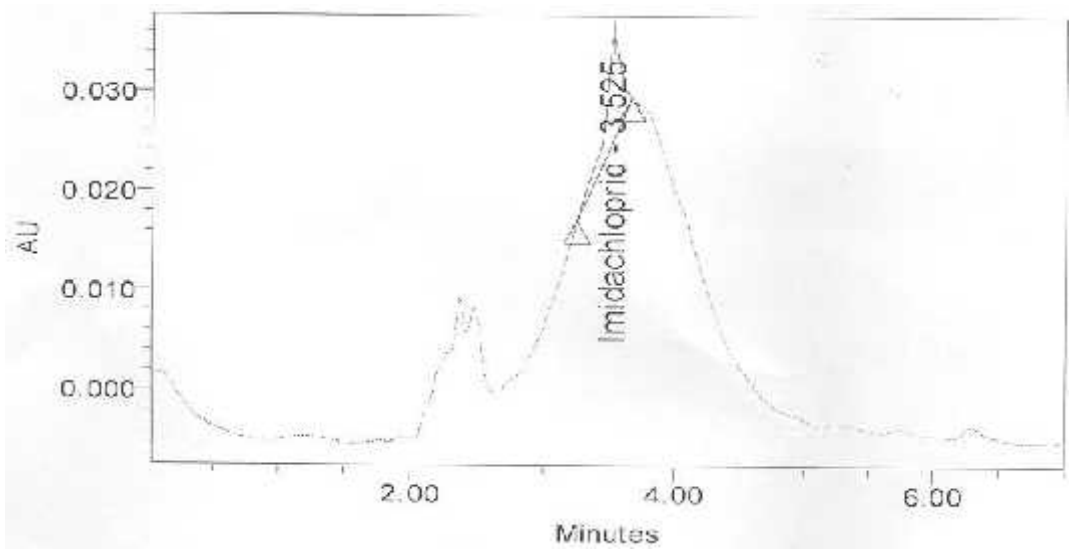


Fig. 1: Calibration curve for various standard solutions of imidacloprid

Table I. Method validation data, Recovery and Precision

Fortification levels	Mean Recovery ± SE †	SD ‡	RSD §
µg g^{-1} rice	**%**	**%**	**%**
0.01	86 ± 6.12	10.6	12.37
0.05	115 ± 3.53	6.11	5.32
0.1	108 ± 6.01	10.41	9.61
0.5	93 ± 3.53	6.11	6.59
1.0	93 ± 3.84	6.66	7.19

† n = 3 ‡ Standard deviation § Relative standard deviation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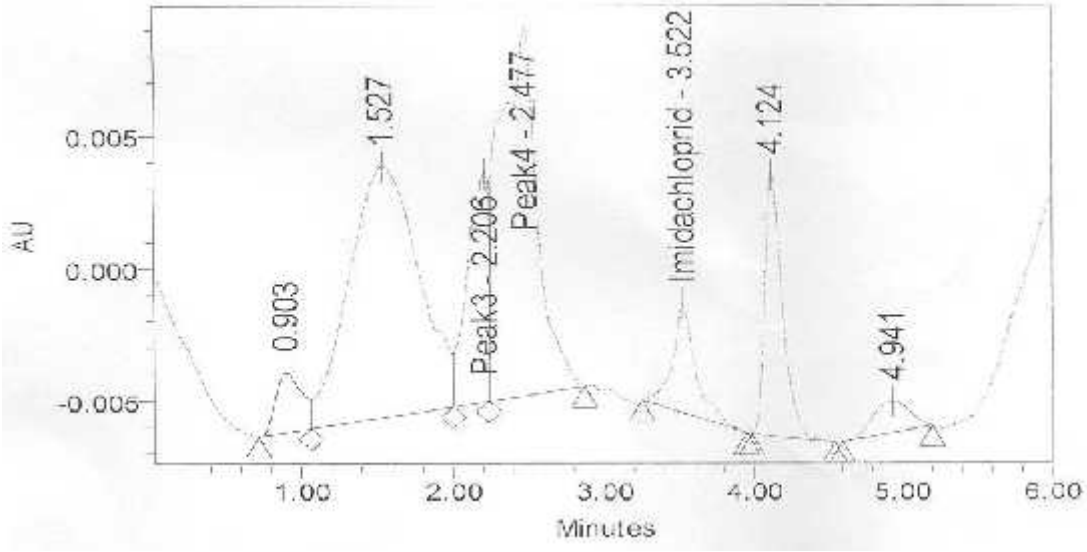
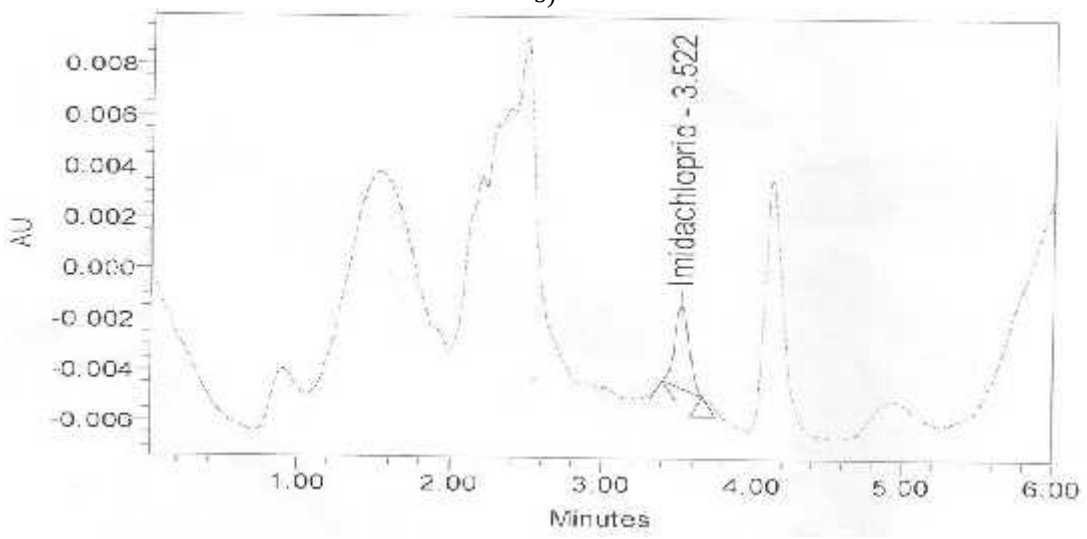
0.020
0.010
0.000
AU
Imidachlopric - 3.525
2.00
4.00
6.00
Minutes

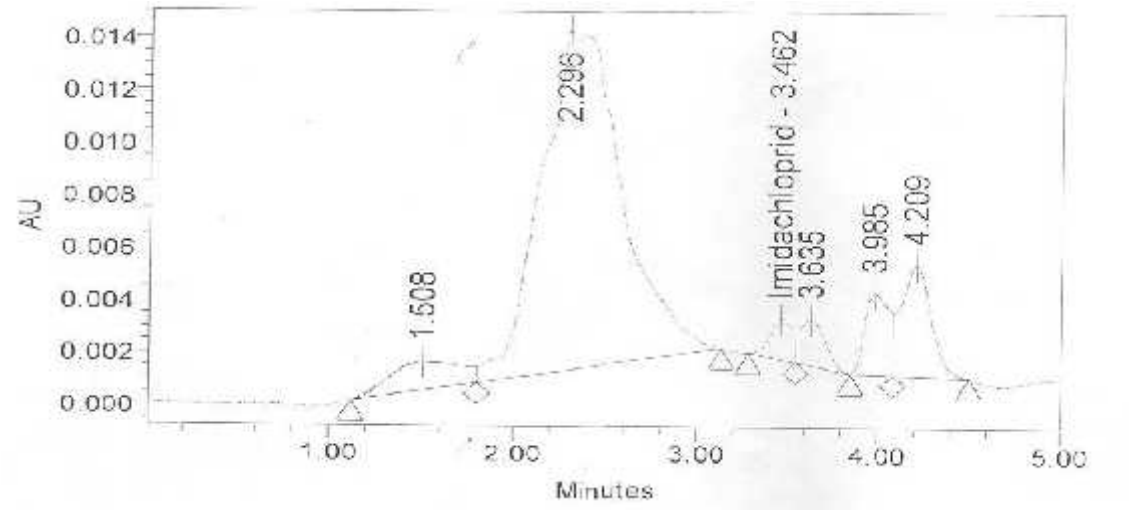
a)


0.005
0.000
-0.005
AU
0.903
1.527
Peak3 - 2.206
Peak4 - 2.477
Imidachloprid - 3.522
4.124
4.941
1.00
2.00
3.00
4.00
5.00
6.00
Minutes

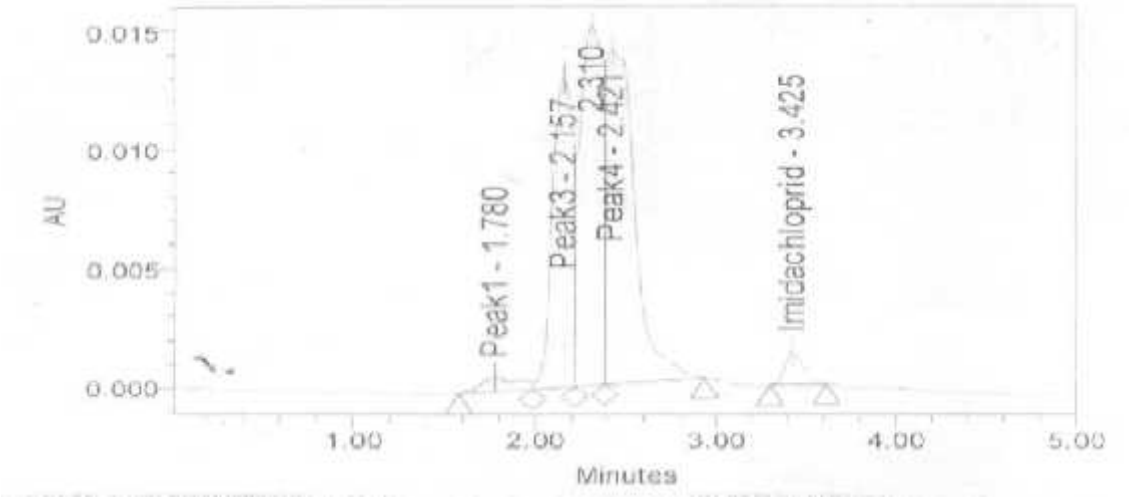
b)


0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
-0.006
AU
Imidachlopric - 3.522
1.00
2.00
3.00
4.00
5.00
6.00
Minutes

c)



d)



e)

Fig. 2: Chromatograms of rice samples spiked with imidacloprid, a) spiking level=1 µg g^{-1} rice, b) spiking level=0.5 µg g^{-1} rice, c) spiking level=0.1 µg g^{-1} rice, d) spiking level=0.05 µg g^{-1} rice, e) spiking level=0.01 µg g^{-1} rice

Table II. Residue range, mean residue level and frequencies of imidacloprid residues in rice samples from various districts of Punjab, Pakistan.

Rice Areas	Residue range	Mean residue level	No of samples		Samples with residue more than MRL †
			Analyzed	Detected	
	µg g^{-1} rice				
Gujranwala	0.47-2.63	0.88 ± 0.31	10	7	1 (2.63) ‡
Sialkot	0.95-2.74	1.5 ± 0.39	10	8	3 (1.63, 2.22, 2.74)
Hafizabad	<0.01-1.16	0.57 ± 0.28	10	6	0
Narowal	0.43-1.88	0.83 ± 0.27	10	5	1 (1.88)
Sheikhupura	0.01-0.82	0.31 ± 0.14	10	5	0
Mandi Bahaudin	0.07-0.76	0.48 ± 0.21	10	3	0
Pasrur	<0.01-0.14	0.06 ± 0.01	10	5	0
Jhang	<0.01-0.1	0.08 ± 0.02	10	4	0
Gross total			**80**	**43**	**5**

† Revised EU maximum residue limit of 1.5 µg g^{-1} rice

‡ Values in parenthesis show actual residue level above MRL in µg g^{-1}

DISCUSSION

Nowadays, highly sophisticated and precious instruments like GCMS and LCMS are used to improve sensitivity and reliability of screening and quantification analysis work. Nevertheless, conventional HPLC when coupled with a suitable and validated method could be more cost effective for routine pesticide residue determination programs (Tuan *et al*., 2009). Some pesticides are especially HPLC compatible ones like insecticide imidacloprid (Singh *et al*., 2008).

Our recovery and relative standard deviation (RSD) values are satisfactory as according to Tuan *et al*. (2009), over 80% are high enough recoveries and <20% are low enough the relative standard deviations for a method to be satisfactorily validated. Results for accuracy of our method are in line with those of Amadeo *et al*. (1996) who reported recovery rates for imidacloprid as 114, 123 and 102% in tomato, pepper and cucumber fruits, respectively. Similarly, Eiki *et al*. (2004) recorded the average recovery as 113.3, 88.0, 82.7 and 87.5% for imidacloprid in cucumber, eggplant, lettuce and green pepper, respectively. Alfonso *et al*. (2006) found the average recovery of imidacloprid to be 73 and 102 % for 0.1 and 1.0 µg g^{-1}, respectively, in peach, pear, courgette, celery and apricot. Kapoor *et al*. (2014) found recovery of imidacloprid ranging from 77.5 to 111% while relative standard deviation from 5.22 to 14.20% against spiking levels of 0.0625, 0.125, 0.25, and 0.50 µg g^{-1}. Nasr *et al*. (2014) recorded the recovery percentage of imidacloprid as 115.92% and 119.1% for spiking levels of 25 and 50 µg.

Use of imidacloprid is very common and its residues in numerous crops have been reported recently. Chauhan *et al*. (2013) determined imidacloprid residues in potatoes. The calculated concentration (0.35 µg g^{-1}) was very much near to the maximum residual limit (0.5 µg g^{-1}). They also find that even after washing with plenty of water, the potatoes were carrying residues of imidacloprid which could surely be lethal for the consumers. Munawar and Hameed (2013) determined high levels of imidacloprid residues in different vegetables collected from six major vegetable markets of Lahore, Pakistan. Actually 70% of the samples collected were found contaminated with imidacloprid. Similarly, Nasr *et al*. (2014) has already determined imidacloprid residues higher than the MRL in cucumber, pepper and tomato.

In rice tract of Punjab, imidacloprid is extensively used as a standard insecticide in spite of the fact that imidacloprid use on rice is strictly prohibited by Rice Research institute, Kala Shah Kaku, Lahore, Pakistan. In rice comparatively longer half-life of imidacloprid has already been reported by Nasr *et al*. (2014) and this could be ascribed to the fact that rice plants would be absorbing imidacloprid from application zone for longer period of times continuously. The determination of imidacloprid residues in rice samples was planned on recommendation of Directorate of Entomology, Faisalabad as pesticide residues in rice of Pakistan is, currently, a serious problem regarding food safety and the export to modern international markets.

Presence of imidacloprid residues in detectable concentrations in various plant tissues such as grains /seeds, leaves and vascular fluids could be ascribed to the systemic nature with such physicochemical properties that allow its residues to enter into plant body and then move throughout the plant using xylem transport and the translamilar (between leaf surfaces) movement (Nasr *et al*., 2014). Likewise, according to Chauhan *et al*. (2013), owing to its high water solubility, imidacloprid finds very high molecular mobility inside xylem of target plants. Moreover, application timing of imidacloprid usually coincides with that of crop maturity, so, direct application at this time could be the reason of its persistence inside the plant tissues as residues in the harvest.

Conclusion: Results of current study show that rice samples from major rice growing areas of Punjab carry high imidacloprid residues. Rice is the staple food and major export to the European markets. Pesticide residues in rice could adversely affect human health and moreover, rejection of shipments from importing countries because of high pesticide residues could not only bring economic loss but also the ill name for the country as well. The information could be useful for policy makers, rice exporters and the public at large.

REFERENCES

Alfonso, D.M., P. Fidente, D.A. Barbini, R. Dommarcoa and S. Seccia (2006). Application of solid-phase extraction and liquid chromatography–mass spectrometry to the determination of neonicotinoid pesticide residues in fruit and vegetables. J. Chromatography A. 1108: 1-6.

Alsayeda, H., S. Pascal-Lorber, C. Nallanthigal, L. Debrauwer and F. Laurent (2008). Transfer of the insecticide [14C] imidacloprid from soil to tomato plants. Environ. Chem. Lett. 6: 229-234.

Amadeo, R.A., V.A. Fernandez-Alba, A.B. Antonio, C. Ana and B. Khalf-Allah (1996). Determination of imidacloprid in vegetables by high performance liquid chromatography with diode-array detection. J. Chromatography. A. 721: 97-105.

Anastassiades, M., S.J. Lehotay, D. Stajnbaher and F.J. Schenck (2003). Fast and easy multiresidue method employing acetonitrile extraction/ partitioning and “dispersive solid-phase extraction” for the determination of pesticide

residues in produce. J. AOAC. Int. 86(2): 412-431.

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

Chauhan, S.S., S. Agrawal and A. Srivastava1 (2013). Effect of imidacloprid insecticide residue on biochemical parameters in potatoes and its estimation by HPLC. Asian J. Pharm. Clin. Res.6(3): 114-117.

Eiki, W.K., H. Baba, T. Eun, Y. Arao and S. Ishii (2004). Rapid and simple screening analysis for residual imidacloprid in agricultural products with commercially available ELISA. Ana. Chimica. Acta. 521: 45-51.

European Food Safety Authority (2010). Modification of the existing MRLs for imidacloprid in rice. EFSA J. 8(4): 1589.

Gupta, L., A. Sharma and A. Shanker (2008). Dissipation of imidacloprid in orthodox tea and its transfer from made tea to infusion. Food Chem. 106: 158-164.

Hladik, M.L. and M.M. McWayne (2012). Methods of analysis- determination of pesticides in sediment using gas chromatography/mass spectrometry: U.S. Geological Survey Techniques and Methods 5-C3, 18 p. Available at http://pubs.usgs.gov/tm/tm5c3.

Hassanzadeh, N., A.E. Sari and N. Bahramifar (2012). Dissipation of imidacloprid in greenhouse cucumbers at single and double dosages spraying. J. Agr. Sci. Tech. 14: 557-564.

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

Kapoor, U., M.K. Srivastava, A.K. Srivastava, D.K. Patel, V. Garg and L.P. Srivastava (2014). Analysis of imidacloprid residues in fruits, vegetables, cereals, fruit juices, and baby foods and daily intake estimation in and around Lucknow, India. Environ. Toxic. Chem. DOI 10.1002/etc.2104

Munawar, A. and S.W. Hameed (2013). Quantification of pesticide residues in vegetables by different chromatographic techniques. J. Chromatograph Separat. Techniq. 4(8) 200. doi:10.4172/2157-7064.1000200.

Nasr, H.M., M.A. Abbassy, M.A. Marzouk and A.S. Mansy (2014). Determination of imidacloprid and tetraconazol residues in cucumber fruits. J. Pollut. Eff. Cont. 2(1): 1-5.

Sanyal, N., D. Hazra, R. Pal, A.K. Somchaudhury and A. Chowdhury (2006). Imidacloprid in processed tea and tea liquor. J. Zhejiang Univ. Sci.B. 7(8): 619-622.

Singh, S.B., I. Mukherjee, J. Maisnam, P. Kumar, M. Gopal and G. Kulshrestha (2008). Determination of pesticide residues in IPM and non-IPM samples of mango (*Mangiferaindica*). J. Environ. Sci. Health Part B.43: 300-306.

Tahir, M. U., S. I. Naik, S. Rehman and M. Shahzad (2009). A quantitative analysis for the toxic pesticide residues in marketed fruits and vegetables in Lahore, Pakistan. Biomedica. 25: 171-174.

Tuan, S., H. Tsai, S. Hsu and H. Li (2009). Multiresidue analysis of 176 pesticides and metabolites in pre-harvested fruits and vegetables for ensuring food safety by gas chromatography and high performance liquid chromatography. J. Food Drug Anal. 7(3): 163-177.