

Original Research Article

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## Development of Modified QuEChERS Methodology Coupled with RP-HPLC for Quantification of Various Insecticides in Rice Grains

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### ABSTRACT

#### Keywords

Imidacloprid, Carbofuran,  
Rice grains, HPLC

#### Article Info

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A method of reverse phase-high performance liquid chromatography (HPLC) was standardized and developed coupled with modified UV-VIS detection system was standardized for simultaneous determination of residues of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin in rice grains. Extraction and clean-up process was done by modified QuEChERS method. The Brownlee Analytical C18 column was used for separation with an isocratic flow @ 0.5 mL min<sup>-1</sup> of mobile phase of acetonitrile and water (90:10, v/v) was operated. The peaks of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and Cypermethrin A, B and C in chromatograms depicted at retention times of 2.67 min, 3.25 min, 3.54 min, 3.93 min, 4.08 min, 8.19 min and 9.32, 9.78 and 10.00 mins respectively. Constant recoveries were observed above 80 % for all the three insecticides when samples were fortified at different levels of 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg<sup>-1</sup> levels.

### Introduction

Being India secured second position in production and consumption of rice in the world next after China. Rice cultivation requires good irrigation facility or heavy annual rainfall. It suits on rainfed areas with hot and humid climate (Barrion *et al.*, 2007). Rice production is mainly affected by various abiotic and biotic factors, which ultimately led to the decline in yield (Behura *et al.*, 2011). Biotic factors include various insect pests which caused significant damage and crop losses. Major biotic factors that influence rice production in the northeastern hills are diseases, insect pests, rodents and weeds.

More than 100 insect species are known to attack the rice crop of which 20 species are considered highly important that results in economic damage (Arora and Dhaliwal, 1996). In Manipur, among the pyralid pests, the leaf folder, yellow stem borer plays a major role in reduction of yield (Barwal *et al.*, 1994).

Starting from early germination to crop maturity stage, around ten species of insects like Gall midge (*Orseolia oryzae* Wood Mason), Yellow stem borer (*Scirpophaga incertulas* Walker), Leaf folder (*Cnaphalocrocis medinalis* Guenee) Rice case worm (*Nymphula depunctalis* Guenee), Rice whorl maggot (*Hyderellia philippina* Ferina),

Small grasshopper (*Oxya chinensis* Walker), Green leaf hopper (*Nephotettix virescens* Distant), Rice gundhi bug (*Leptocorisa acuta* Thunb), Tobacco caterpillar (*Spodoptera mauritia* Boisid) and Rice army worm (*Mythima seperata* Walker) were reported to be the most important and regular pest of this crop in Manipur. Different species of stem borer (*S. incertulas*, *C. suppressalis* and *Sesamia inferens*), Gall midge (*Oreoseolia oryzae*), leaf folder (*C. medinalis*), case worm (*N. depunctalis*) and plant hoppers (*Nilaparvata lugens* and *Sogatella furcifera*) are the major pests causing severe damage to rice crops (Jenita *et al.*, 2016). Farmers use various groups of insecticides to combat the damage caused by insect pests. Most of the farmers use insecticides like monocrotophos, dichlorvos, malathion, chloropyrifos, Cypermethrin, deltamethrin, thiamethoxam, Imidacloprid, carbofuran, chlorantraniliprole, flubendiamide, fipronil, etc. Ideally a pesticide must be lethal to the targeted pests only, but not to non-target species, including human being. Unluckily, this is not happened, so the storm of use and abuse of pesticides has risen up. Pesticides can contaminate soil, water, turf, and other vegetation. Once the pesticides have applied, they can harm plants and animals ranging from beneficial soil microorganisms and insects, non-target plants, fish, birds, and other wildlife (Aktar *et al.*, 2009). There are no informations available on the nature and quantity of pharmacologically active compounds of different insecticides in rice which is necessary to ensure the safety of the consumers and the environment. Therefore, the method was developed for the estimation of various insecticides in rice.

## Materials and Methods

The experiment was conducted at Indian Council of Agricultural Research-Research Complex for North Eastern Hill region Manipur Centre, Lamphelpat, India.

The technical grade analytical standards such as Imidacloprid (99.9%), carbofuran (98.0%), chlorantraniliprole (99.20%), fipronil (97.5%), malathion (98.0%), chloropyrifos (98.0%) and cypermethrin (94.3%) and primary secondary amine (PSA) sorbent were procured from sigma-aldrich, Kolkatta (Fig. 1). Chemicals such as sodium chloride, activated anhydrous magnesium sulphate (MgSO<sub>4</sub>), anhydrous sodium sulfate and solvents like high-performance liquid chromatography (HPLC) grade acetonitrile, HPLC grade water were obtained from E. Merck (India) Ltd, Mumbai, India. All the solvents used were of laboratory grade and were redistilled in all glass apparatuses before experiment. The suitability of the solvents and other chemicals was ensured by running reagent blanks before real analysis.

The high-performance liquid chromatograph (series 200) was equipped with a reverse-phase, RP, C18 column and a UV-VIS detector, and dual pumps supplied by M/S Perkin Elmer, United States. The HPLC column, a Brownlee Analytical C18 column (150 mm column length, 4.6 mm inside diameter and 5 µm particle size) was also procured from M/S Perkin Elmer. For the control of instrument, data acquisition and processing, TC Nav software was used. A good satisfactory separation of peak symmetry was obtained with an isocratic mobile phase comprising of acetonitrile: water (90: 10, v/v) at a flow rate of 0.5 mL/min. Quantification was achieved with UV-VIS detection at 225 nm based on peak area with a retention factor of 10 min and injection volume of 20 µL.

A standard stock solution of thiamethoxam, flubendiamide and endosulphan (1 mg/mL) was prepared in HPLC grade acetonitrile. For the construction of a calibration curve (2.00, 5.00, 10.00, 15.00 and 20.00 mg/mL) the standard solutions required were prepared from stock solution by serial dilution with

HPLC grade acetonitrile. All standard solutions were stored at 4°C before experiment.

Rice grains were used as substrates for standardization of the methodology proposed for estimation of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin. Rice grains treated to be control samples were collected from hill-top Senapati district (Manipur) without any history of application of pesticides. The rice grains samples were fortified at different levels, i.e., 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg. There were five replications for each treatment.

The quick easy cheap effective rugged and safe (QuEChERS) method was modified by taking a representative sample of 15 g of rice grains was weighed into a 50 mL centrifuge tube. After that 30 mL of acetonitrile was poured into all the centrifuge tubes. The samples were vigorously shaken and homogenized at 15,000 rpm for 2–3 min using a Heidolph homogenizer. Sodium chloride (10 g) was added to each sample and shaken dynamically by rotospin for around 5 min. The samples were centrifuged using a laboratory centrifuge for 3 min at 5500 rpm.

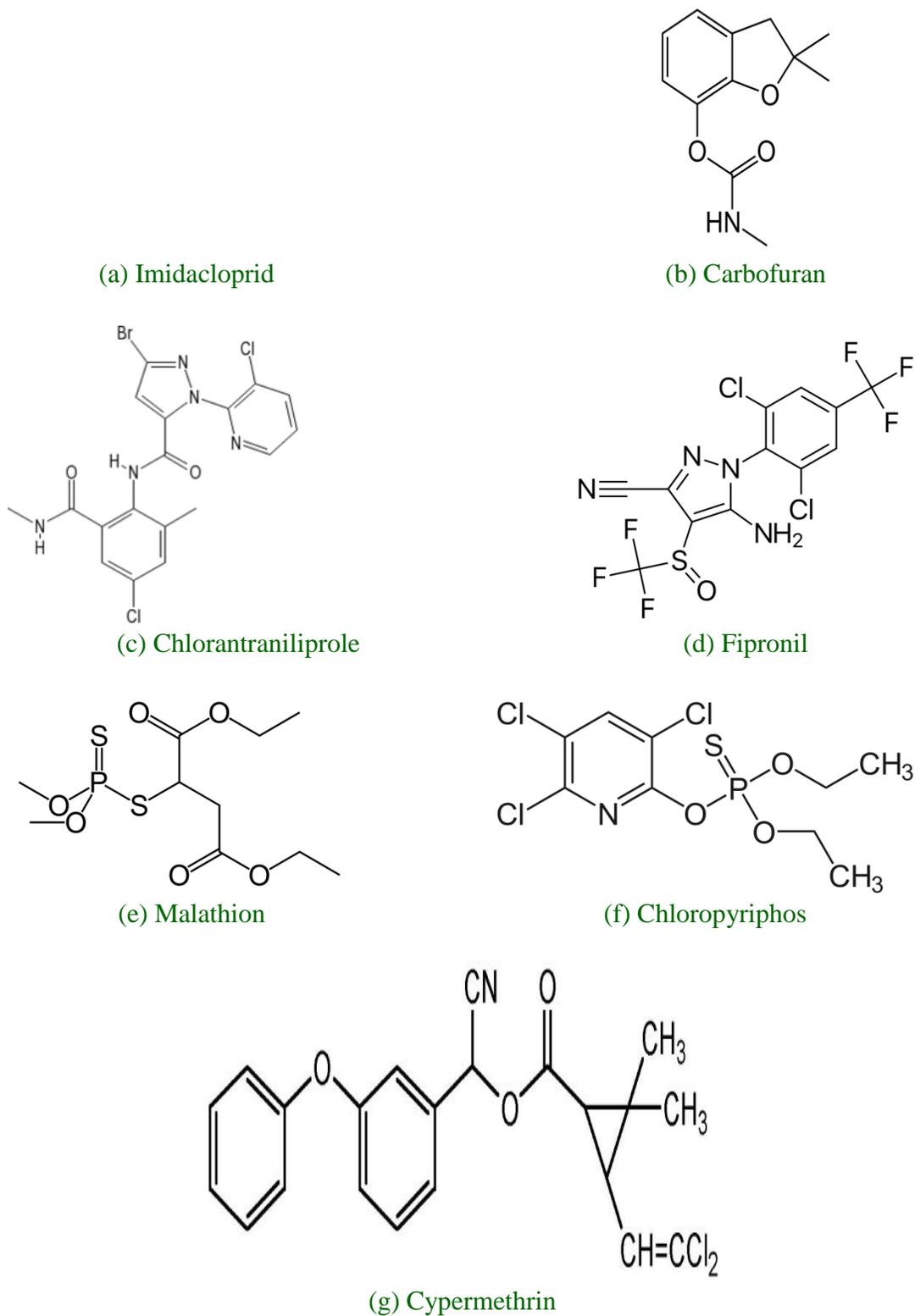
The top 15 mL organic layer from each of the 15 mL tube was decanted into another 50 mL centrifuge tube which was weighed with 10 g of activated sodium sulfate. It was again shaken using a rotospin for 2 min. The sample extract (6 mL) was transferred to a 15 mL centrifuge tube containing PSA sorbent (150 mg) and activated anhydrous magnesium sulfate (900 mg). The tube was tightly capped and vortexed for 30 s. The tubes were centrifuged for 3 min at 2,500 rpm. The top extract (4 mL) was transferred into a test tube and concentrated to 2 mL with a rotary evaporator under 35°C for further quantification by HPLC.

## Results and Discussion

Reversed-phase HPLC, with UV-VIS detection, was shown to be good for determination of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin because no need for derivatization step. Chromatographic separation in C18 columns provides good outcome. The detection at 225 nm provides suitable chromatograms for the quantification of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin in real samples. Under the preferred conditions, imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin showed retention factors of 2.67 min, 3.25 min, 3.54 min, 3.93 min, 4.08 min, 8.19 min and 8.19 min respectively (Fig. 2).

The fortified samples of rice grains at different levels were found to be in the range of 82.12 to 95.74 % for imidacloprid, 87.09 to 93.18 % for carbofuran, 80.25 to 90.18 % for chlorantraniliprole, 80.87 to 97.72 % for fipronil, 84.56 to 90.14 % for malathion, 86.12 to 97.43 % chloropyrifos and 85.90 to 87.78 % for cypermethrin A, cypermethrin B and cypermethrin c were found to be in the range of 82.18 to 88.34 %, 80.90 to 91.95 %, 82.24 to 98.27 % and 86.71 to 97.80 % respectively (Table 1). A specific, simplified, quick, cost-effective reversed-phase HPLC method by using QuEChERS has been developed for the determination of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin. The consistent recoveries ranging from 80.25 to 97.79 % for all the insecticides were observed when samples were fortified at 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg levels. The present QuEChERS method is quite effective and provides the quickest, easy and cheap method as compared with that of the traditional methods.

**Fig.1** Chemical structure of (a) imidacloprid, (b) carbofuran, (c) chlorantraniliprole, (d) fipronil, (e) malathion, (f) chloropyriphos and (g) cypermethrin

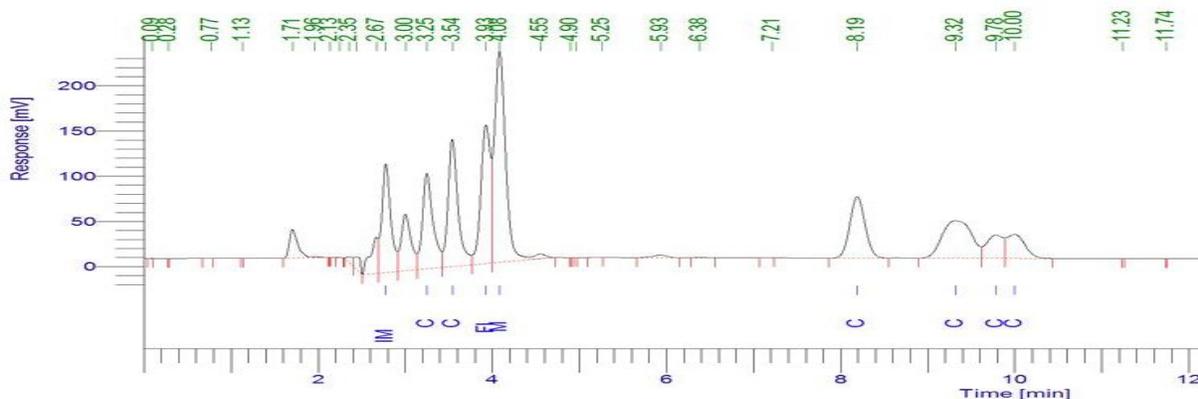


**Table.1** Recovery (%) of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin A, cypermethrin B and cypermethrin C in rice grains (n=5)

Level of fortification (mg kg <sup>-1</sup> )	Imidacloprid	Carbofuran	Chlorantraniliprole	Fipronil	Malathion	Chloropyrifos	Cypermethrin A	Cypermethrin B	Cypermethrin C
0.05	82.12±2.34	87.09±3.02	89.76±1.08	95.87±3.00	87.89±0.67	95.76±1.43	87.78±2.35	90.25±2.65	90.25±2.80
0.10	93.05±1.92	93.18±1.24	90.18±3.01	94.13±1.94	86.44±2.20	86.12±3.25	87.49±1.01	85.07±3.08	90.60±2.31
0.25	86.35±3.80	90.12±2.78	80.25±1.67	80.87±3.89	90.14±1.90	97.43±0.65	87.14±3.06	91.10±1.09	81.15±3.08
0.50	92.12±1.03	87.76±2.67	86.55±3.93	84.00±3.04	84.56±4.67	95.10±1.37	87.65±2.09	91.11±2.00	93.34±2.13
1.00	95.74±2.22	87.70±2.02	89.90±1.08	97.72±1.98	87.25±2.82	95.24±2.79	85.90±1.68	90.55±3.02	97.79±2.23

<sup>a</sup>Mean ± Standard deviation

**Fig.2** HPLC chromatograms of standards of imidacloprid, carbofuran, chlorantraniliprole, fipronil, malathion, chloropyrifos and cypermethrin A, cypermethrin B and cypermethrin C in which retention times on x-axis and % deflection on y-axis



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