METHOD VALIDATION OF QUECHERS AND HPLC-MS/MS IN THE DETECTION AND QUANTIFICATION OF NEONICOTINOIDS IN LOCAL VEGETABLES

Jonathan V. Paz[1,*], Edna C. Mijares[2], Lilibeth DlC. Coo[1]

[1] Institute of Chemistry University of the Philippines, Diliman.
[2]Jefcor Laboratories, Inc.
*Corresponding author: jvpaz@alum.up.edu.ph

ABSTRACT

Numerous methods have been utilized to extract and quantify pesticide residues from various matrices. However, as part of green chemistry goals of minimizing the ecological impact of conducted chemical analysis with a maximum yield of data, the most effective and practical way to analyze pesticides is through multi-residue methods capable of measuring multiple pesticide residues in food from a single extraction. In this study, a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method, coupled with High-Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS) workflow was validated for the extraction and quantification of imidacloprid, thiamethoxam, and dinotefuran in bitter gourd, cabbage, and eggplant. After validation, the method was employed on market samples to determine whether the selected neonicotinoids can be detected and quantified. Method validation determined that the methods were within the acceptance criteria required for single-laboratory method validations. The analytical method showed satisfactory specificity, selectivity, and linearity ($R^2 > 0.99$). Recoveries were within the acceptance criteria of 70-120% and relative standard deviation below 20% at three spiking concentrations: 0.01 µg/g, 0.05 µg/g, and 0.10 µg/g. Random market basket samples during the study have identified an exceedance above 0.01 µg/g for imidacloprid in eggplant.

Keywords: QuEChERS, HPLC-MS/MS, pesticide residue analysis, neonicotinoids, method validation

INTRODUCTION

Neonicotinoids, a class of insecticides developed in the 1980s and first registered in the mid-1990s, are now one of the important insecticides introduced since the pyrethroids (Chen 2014; Cimino et al. 2017; Tomlin 2006; Jeschke, 2011). Neonicotinoids are among the emerging pesticides used in the

Philippines, owing to their effective control of chewing and sucking insects, along with the expiration of data protection clauses under local regulations.

Today, more stringent residue limits are being applied to pesticides internationally. The need for improved pesticide quantitation methods, not only in terms of detection sensitivity but also in terms of reduction of chemical hazards for the analysts and the environmental impact of the analysis itself, is needed to augment regulatory agencies and research institutions. Today, sensitive instruments such as high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) have become the most widely accepted technique and are indispensable for quantification by food safety regulators and research institutions alike (Anastassiades, 2003 & 2007). The availability of pesticide analytical methods suitable for old and new pesticide chemistries allows for the rapid determination and quantification, allowing for decision makers to adapt and apply Integrated Pest Management (IPM) practices, ultimately minimizing pesticide use while achieving effective pest control and protection of the environment and human health.

This study aimed to validate a method for the extraction and cleanup of neonicotinoid residues in vegetables utilizing QuEChERs and the separation and quantitation using LC-MS/MS techniques that can be implemented for local pesticide residue monitoring by nationally accredited pesticide laboratories for improved pest management practices and safer food production. Specifically, the study aimed to: 1) expand the application of the QuECHERs method in the analysis of neonicotinoid residues to new vegetable matrices: bitter gourd, cabbage, and eggplant; 2) apply the HPLC-MS/MS method for the detection and quantification of the three neonicotinoids of interest in these new matrices; 3) validate and determine the methods' "fitness for purpose" according to international guidelines for the determination of residues in food; and apply the validated procedure in the analysis of local market basket samples.

Limitations during the study include the number of sampling sites for market basket samples, as there were quarantine protocols limiting mobility for sampling.

MATERIALS AND METHODS

Chemicals and Other Reagents

Dinotefuran, Imidacloprid, and Thiamethoxam analytical standards utilized were from the standards repository of Jefcor Laboratories, Inc. The purities of all standards used were higher than or equal to 99.7%. These reference standards were stored following specifications stated under the respective certificates of analysis. QuEChERS salt kits and dispersive SPE cleanup kits were availed from Agilent Technologies (Santa Clara, CA, USA). LC-grade and LC-MS-grade acetonitrile were availed from Merck Millipore (Darmstadt, Germany).

Vegetable Collection & Sample Preparation

The control samples for this study were locally certified organic vegetables purchased from a supermarket. Market basket samples were randomly collected within the vicinity of Dasmariñas City, Cavite. A minimum of one kilogram or five pieces of vegetables were collected from the respective sampling site.

Upon collection, the vegetables are placed in a polyethylene sampling bag for storage. The samples are stored in iceboxes (1-5 ℃) and transported immediately to the laboratory for processing. Vegetable samples were processed using a high-powered blender to achieve a homogeneous sample. The analytical portion of each sample was stored in a resealable zipper storage bag, labeled, and frozen (-20 ±1 ℃) until further analysis to prevent degradation.

Preparation of Spiked Sample

Organic vegetables were used as the control samples in the recovery analysis. The required amount of sample (10 g) was weighed into a 50ml polypropylene tube and 0.5 ml of the spiking solution was added to each sample. Spiked samples were vortex-mixed for a minute and left undisturbed for 30 minutes. After the settling time, the samples are extracted according to the protocol being validated.

Sample Extraction and Cleanup

Sample extraction was done according to the modified QuEChERS protocol based on the AOAC Method (AOAC, 2007) outlined below:

Ten (10) grams of the homogenized sample is placed into a 50ml polypropylene centrifuge tube. 10 ml of 1% (v/v) acetic acid in acetonitrile was added and vortex-mixed for one minute, then mechanically shaken. After shaking, 6g anhydrous magnesium sulfate and 1.5g anhydrous sodium acetate were added. The mixture is shaken immediately by hand, followed by mechanical shaking. After shaking, the tubes are vortex-mixed for 30 seconds before centrifugation at 3000 rpm. For the sample cleanup, 8 mL aliquots of the acetonitrile layer were transferred by pipette to a 15-dispersive SPE tube containing primary-secondary amine (PSA) and magnesium sulfate (1200mg + 400 mg). The tube was vortex-mixed and then centrifuged at 3000 rpm. An aliquot of the supernatant was filtered through a 0.2 µm nylon membrane filter. The filtrate was collected in a 2 mL amber autosampler vial, ready for injection into the LC-MS/MS.

Preparation of Matrix-Matched diluents and calibration solutions

Control samples were processed according to the extraction and cleanup procedure above. The extracts from each procedure were pooled and used as the diluent in preparing the matrix-matched calibration standards instead of diluting using the neat extracting solvent. Nine (9) concentration levels were prepared for each matrix, which was the same concentration of the calibration solution dissolved in the extraction solvent.

Storage of Extracts and Calibration Solutions

When solutions were not injected immediately into the LC-MS/MS, the solutions were stored in the freezer at -20 ± 1 °C.

Chromatographic conditions

Chromatographic separations were carried out using an Agilent 1200 Series LC system (Agilent Technologies, Waldbronn, Germany) high-performance liquid chromatograph coupled to an electrospray ionization-triple quadrupole mass spectrometer (HPLC-MS/MS). Analyte separation was achieved using an Agilent Poroshell 120 C_{18} column (50 mm × 2.1 mm, 3 µm particle size) with an

HPLC Gradient with acidified water and acetonitrile. The LC Chromatographic conditions are outlined in **Table 1.**

Target compounds are detected and quantified with an Agilent Technologies 6470 Triple quadrupole LCMS equipped with Agilent Jet Stream Technology (AJS) – heated electrospray ionization. The optimized ionization method is presented in **Table 2.**

Table 1. Liquid chromatograph operating parameters.

Solvent A	0.1 % Formic acid in water
Solvent B	0.1 % Formic acid in acetonitrile
Seal & needle wash	Methanol: H_2O 75:25 (v/v)
Column oven temperature, ºC	40
Injection volume, µL	10
Flow rate, mL/min	0.25
Stop time, min	7.5
Post time, min	5

Table 2. Mass spectrometer operating parameters for nitrogen as collision and drying gas.

Parameter	**Value**
Gas Temperature, ºC	250
Gas Flow, L/min	5
Nebulizer Pressure, PSI	30
Sheath gas heater, ºC	200
Sheath gas flow, L/min	7
Capillary voltage, V	5000
$V_{charging}$, V	500
Scan type	MRM
Dwell time, msec	200

The instrument was operated in positive ion mode with multiple reaction monitoring (MRM). In this study, the most intense and characteristic MRM

transitions were selected for each analyte, and the more intense production was used for quantitation. The selected MRM transition for each analyte is presented in **Table 3.**

Analyte quantification was based on the peak area of each analyte, which is within the retention time window of ± 0.2 minutes. Aside from the retention time, Analytes were identified using their product ion ratio, where the abundances of the daughter ions must be within ± 30% of the parent ion ratio, compared to the ionization of calibration solutions in the solvent.

Table 3. Multiple reaction monitoring conditions.

Compound	Precursor Q1 Mass (AMU)	Product Q3 Mass (AMU)	Fragmentation Voltage (V)	Collision energy (eV)
Imidacloprid	256	208.9	80	12
		175		12
Thiamethoxam	292	211.1	85	8
		181.1		20
Dinotefuran	203	129	100	14
		113		14

Calibration and Sample Analysis

External calibration was used in this study. Standards were injected at the start, in between, and at the end of the sequence run to check for potential signal drift. The concentration range of the calibration solution encompassed the equivalent concentration of the analytes from 0.5x LOQ to 100x LOQ. To further minimize carryover, solvents were injected before and after the injections of the calibration standards and samples.

The concentrations of the analyte were calculated as follows:

$$mg/kg\ (\mu g/g, ppm) = \frac{\frac{Response - (y - intercept)}{slope}\ x\ TV, mL\ x\ DF}{Sample\ Weight\ , g}$$

Equation 1. External Calibration Calculation

Where:
DF = dilution factor
TV = Total extract volume

Method validation

The method validation was by extraction and quantitation of neonicotinoids from fortified vegetable matrices. The approach follows the guidance document for single laboratory method validation of the Codex Alimentarius Commission and the European Union (CXG 90-2017; SANCO/10684/2009; SANTE 12862/2019; Thompson et al. 2002).

The performance parameters assessed include the following:

Selectivity – The selectivity of the method for the analytes was determined by comparing the slope and the instrument response for each analyte dissolved in extracting solvent and in the matrix- matched calibration solutions.

Calibration and Linearity - Linearity was determined through the plot of residuals produced by linear regression of the responses with the concentrations in the calibration set.

Matrix Effects - To determine the significance of the matrix effect on the instrument response, matrix-matched calibrations were prepared. The instrument responses of the analyte in solvent-only and in the matrix blank extracts were compared to determine the matrix effect.

The significance of the matrix effect was evaluated by the student's T-test. Secondary verification of the matrix effect is calculated from the peak area of the standard in the solvent ($PA_{solvent}$) and the peak area of the standard dissolved in the matrix-match calibration (PA_{mmc}). A significant matrix effect is considered above ± 30%.

$$\%\text{Matrix Effect} = \frac{(PAsolvent - PAmmc)}{PAsolvent} x\ 100\%$$

Equation 2. Determination of Matrix Effect

Accuracy - To demonstrate that the method can provide accurate and precise results at a range of concentrations, a recovery analysis was conducted. The recovery analysis was done by fortifying control samples with a known amount of neonicotinoid at the targeted LOQ, 10x targeted LOQ, and 50x targeted LOQ. The recovery values of the fortified samples were calculated using the formula below and reported to the nearest whole number percentage.

$$\boldsymbol{Percent\ Recovery}, \% = \frac{Actual\ Residue\ Level, mg/kg}{Fortification\ Level, mg/kg}\ x\ 100$$

Equation 3. Determination of Recovery

The method is deemed accurate if the results of the recovery analysis at the targeted LOQ and 10x targeted LOQ are within 70% - 120% for

and 70% - 110% for samples fortified at 50x the targeted LOQ.

Precision – The precision of the method is based on the calculation of the relative standard deviation (RSD). The method is precise if the relative standard deviation for the replicates at each fortification level is ≤ 20%

Limit of Quantitation - The defined limit of quantitation (LOQ) for pesticide residue analysis is the lowest validated spike concentration that demonstrates an accuracy of 70-120% and precision of ≤20% and has a signal-to-noise ratio of ≥ 10.

Market Basket Sample Analysis

Grab (random) vegetable samples purchased from the vicinity of Dasmariñas City, Cavite, were analyzed following the developed method. Limitations attributed to the COVID-19 quarantine protocols have limited the sampling to locations within the vicinity of Dasmariñas Cavite.

RESULTS AND DISCUSSION

To meet the growing demands for a reliable, cost-effective, analyst and, environmentally friendly, and accurate pesticide analysis protocol, it is important to establish and implement analytical methods that adhere to internationally accepted method validation guidelines. The methods selected for method validation incorporated the optimized extraction procedure and were validated to determine their performance using the assessment parameters for single laboratory method validation: performance characteristics such as selectivity, linearity, trueness, precision, limit of quantification, and matrix effects.

Analyte Identification and Method Specificity/Selectivity

The developed method allows the resolution of dinotefuran, imidacloprid, and thiamethoxam using a liquid chromatograph with a tandem mass spectrometer detector. Common interferences were checked by analyzing reagent/method blanks for every batch of reagents and samples. The reagent/method blanks, together with the matrix blank/unfortified control samples, were extracted and cleaned up according to the analytical procedure. Analyzed method blanks showed that they were free from interferences at the expected retention times and responses (<20%) compared to samples fortified at

LOQ. The responses of the untreated or control samples were similarly free from interferences at the expected retention times and responses (<30) of the analytes at LOQ (**Figures 1** and **2**). The use of a tandem MS/MS as the detector offers high selectivity in the detection of the analytes of interest. The identities of analytes were based on the retention time (±0.2 minutes) of each analyte and confirmed by the ion ratio. The peaks of analytes were within ±30% (relative) of the ion ratio average of the calibration standards prepared in solvent when measured in the same sequence and under the same conditions. The method is therefore considered specific in the determination of the analytes based on the results of the analysis of reagent and matrix blanks and the identification of the analyte at the expected retention times.

Calibration, Linearity, and Drift

The linearity of the detector response to dinotefuran, imidacloprid, and thiamethoxam was determined by external calibration. Calibration standards were prepared in pure solvent and in matrix-matched solutions to determine the matrix effects caused by the matrices. Bracketing calibration was done to determine any significant drift in the response of dinotefuran, imidacloprid, and thiamethoxam throughout the sequence run with acceptable criteria of ≥30%. The system suitability check of the instrument was determined by the signal-to-noise ratio of the analyte at the lowest concentration of the calibration standard in the calibration curve (lowest calibrated level). The signal-to-noise ratio (S/N) at the lowest calibration level was ≥10, as evident in the chromatogram of the standard solutions prepared.

The instrument showed good linearity for dinotefuran, imidacloprid, and thiamethoxam, as presented in **Figure 3A**. The fit of the calibration function was determined by the plot of the residuals produced by the linear regression of the responses on the concentrations, as presented in **Figure 3B**. A correlation study between analyte concentration and the detector response was determined by injecting standards in the extraction solvent at different concentrations, from 0.005 to 1 µg/mL. In general, very good linearity, with correlation coefficients higher than 0.99, was obtained for all analytes

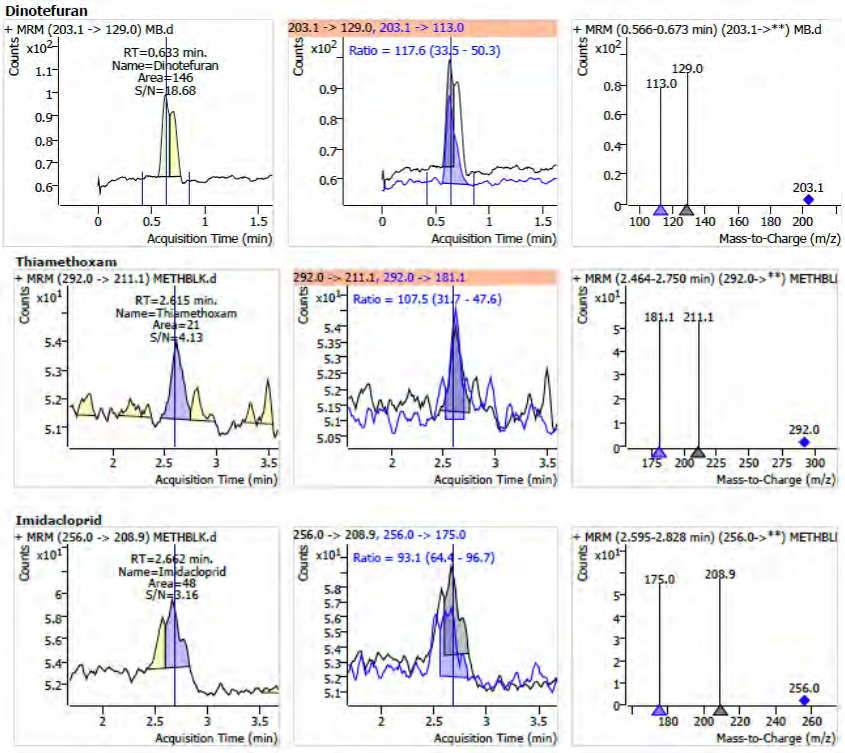

Figure 1. Representative extracted ion chromatograms of the method blanks.

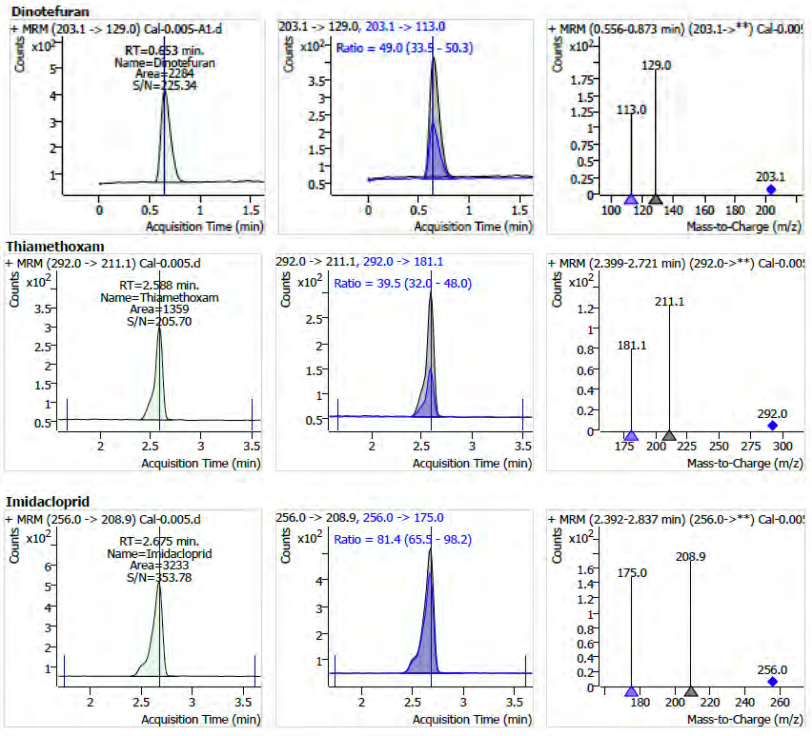

Figure 2. Representative extracted ion chromatograms of analytes at the lowest calibration level.

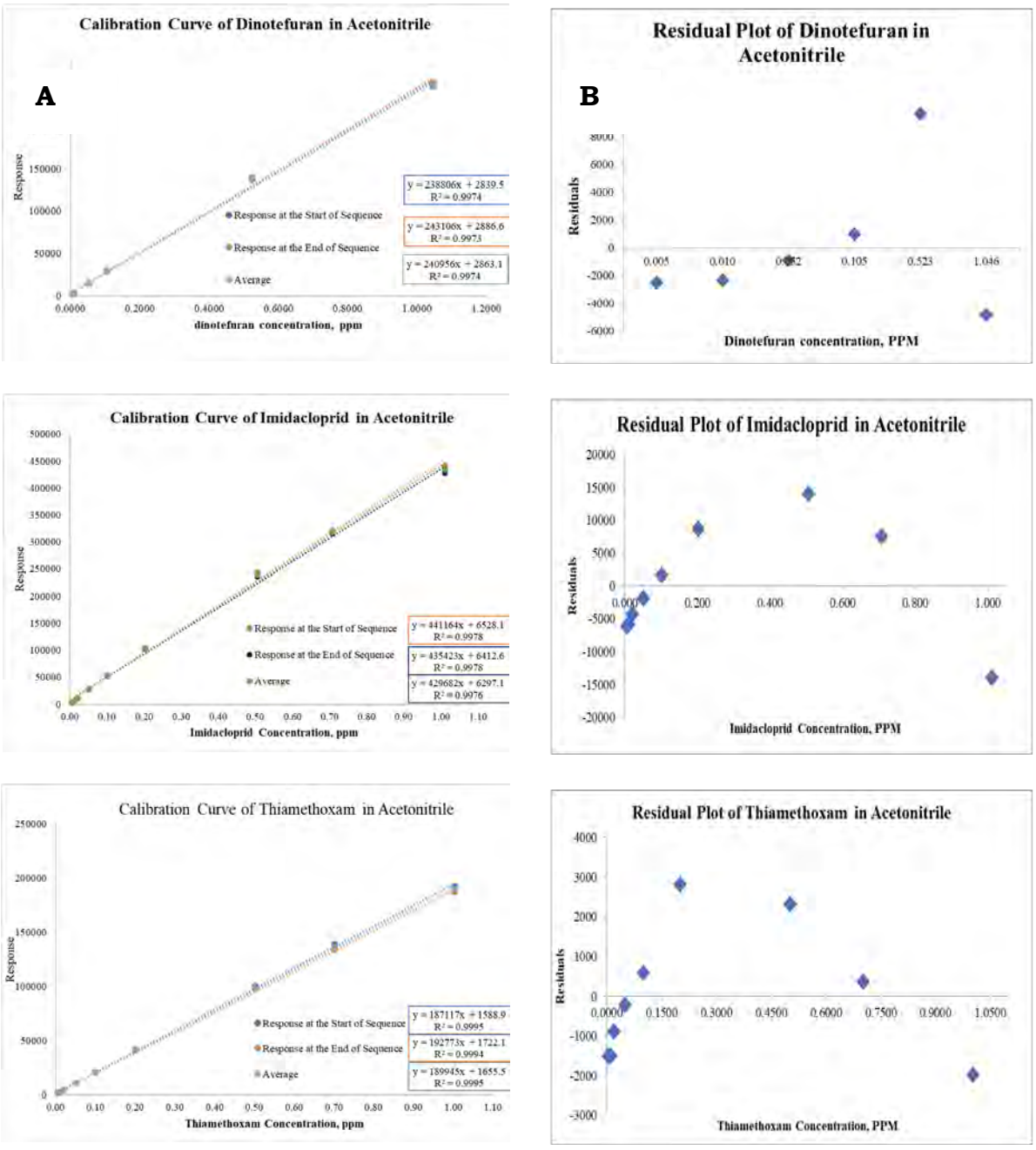

Figure 3. A. Representative calibration curves of analytes prepared in solvent. **B.** Representative residual plots of analytes.

Determination of Matrix Interferences (Matrix Effect)

The use of matrix-matched calibration standards with the (QuEChERS) method was evaluated to address variations in ionization efficiency in the LC–MS/MS determination of neonicotinoid pesticides in complex vegetable samples. The instrument responses of the analyte in solvent-only standard and the matrix blank extracts were compared to determine the matrix effect. To determine the significance of the matrix effect on the instrument response, standards prepared in pure solvent and in matrix-matched solutions were injected, and their responses were compared. The matrix effect is calculated according to the formula for t-test value (tcalc) calculation. A secondary evaluation using the Matrix effect (%ME) calculations, following the EU/SANTE guidelines, is also presented. Matrix effects were classified into different categories based on the value of this percentage. No matrix effect was considered when the %ME values were below ±30%. A representative calculation of the matrix effect for each matrix-analyte combination is presented in **Table 4**.

Table 4. T-test values and percent matrix effect for analytes in solvent and matrix-matched solutions.

Analyte / Matrix	T_{Calc}	% ME
Dinotefuran /Bitter gourd	2.534	59
Dinotefuran /Cabbage	2.561	55
Dinotefuran /Eggplant	1.681	68
Imidacloprid /Bitter gourd	1.948	23
Imidacloprid /Cabbage	1.948	24
Imidacloprid /Eggplant	1.948	6
Thiamethoxam / Bitter gourd	2.256	14
Thiamethoxam / Cabbage	2.108	19
Thiamethoxam / Eggplant	2.108	4

T-limit at n=9 at 95% Confidence interval is 2.306

According to the result of the analysis, the matrix effect on the response of the imidacloprid and thiamethoxam in all matrices was not significant for dinotefuran; however, the matrix effect was evident for all matrices. As such, the quantitation of imidacloprid and thiamethoxam was based on the external calibration curve as dissolved in acetonitrile, while matrix-matched calibration solutions were used for samples containing dinotefuran.

Precision and Trueness

The precision (repeatability) and trueness (recovery) of the methods were evaluated by the analysis of spiked control samples. As determined during the initial method optimization phase, two sets of control samples were prepared: imidacloprid with thiamethoxam and individual dinotefuran. Three spiking levels, 0.01, 0.10, and 0.5 mg/kg with 7, 5, and 3 replicates, were analyzed to determine the reliability of the method within this wide range of concentrations. The recovery for the targeted LOQ for the three neonicotinoids, at 0.01 mg/kg, and the other two spiking concentrations are presented in **Table 5** and have provided satisfactory recovery of being within the acceptable range of 70 to 120 percent, following the performance criteria set by the Codex and EU guidelines for single laboratory method validations.

Table 5. Mean recoveries and relative standard deviation (RSD) of analytes at 1x, 10x, and 50x limit of quantitation (LOQ) spiking levels.

Matrix	Spiking Conc. mg/kg	n	Percent Recovery ± RSD		
			Dinotefuran	Imidacloprid	Thiamethoxam
Bittergourd	0.01	7	100 ± 3	101 ± 6	111 ± 2
	0.10	5	84 ± 4	79 ± 6	82 ± 4
	0.50	3	82 ± 4	72 ± 2	76 ± 3
Cabbage	0.01	7	96 ± 7	88 ± 5	97 ± 4
	0.10	5	94 ± 13	77 ± 4	99 ± 5
	0.50	3	107 ± 3	70 ± 4	75 ± 4
Eggplant	0.01	7	116 ± 9	103 ± 3	109 ± 4
	0.10	5	95 ± 3	92 ± 2	92 ± 2
	0.50	3	94 ± 3	85 ± 2	86 ± 2

Determination of Detection and Quantification Limits

The determination of the limit of detection, and sometimes also the limit of quantification, of a technique is regarded as a very important aspect of method validation as it indicates the range of concentrations over which the technique can reliably be applied. The defined limit of quantification (LOQ) for the analysis is selected as the lowest validated spike concentration that demonstrates

trueness of 70-120 % and precision of ≤20 %. In this study, all matrix-analyte combinations were able to provide the set criterion at the lowest spike concentration of 0.01 mg/Kg; as such, this is the set quantification limit for the study. The summary of method performance characteristics is presented in **Table 6.**

Table 6. Summary of method performance characteristics.

Performance characteristics	**Imidacloprid + Thiamethoxam**			**Dinotefuran**		
	Bittergourd	Cabbage	Eggplant	Bittergourd	Cabbage	Eggplant
Linearity	Linear ($r^2 \geq 0.99$)	Linear ($r^2 \geq 0.99$)	Linear ($r^2 \geq 0.99$)	Linear ($r^2 \geq 0.99$)	Linear ($r^2 \geq 0.99$)	Linear ($r^2 \geq 0.99$)
Linear range, mg/kg	0.005 – 1.0	0.005 – 1.0	0.005 – 1.0	0.005 – 1.0	0.005 – 1.0	0.005 – 1.0
Drift of calibration solutions	< 30%	< 30%	< 30%	< 30%	< 30%	< 30%
Recovery	> 70% < 110%	> 70% < 110%	> 70% < 110%	> 70% < 110%	> 70% < 110%	> 70% < 110%
Precision	RSD <20%	RSD <20%	RSD <20%	RSD <20%	RSD <20%	RSD <20%
Limit of Quantification, mg/kg	0.01	0.01	0.01	0.01	0.01	0.01
Matrix Effects	Not significant	Not significant	Not significant	matrix-matched calibration		

Analysis of Market Basket Samples

After the validation of the analytical methods, several vegetables collected from two local market stalls were analyzed. Samples were collected randomly and were treated as grab samples, with the farm location simply asked of the vendors. Details of the market samples are presented in **Table 7**.

Table 7. Collection sites of market basket samples.

Matrix	Collection Site	Farm location	Laboratory code
Eggplant	FCIE Talipapa	San Pablo, Laguna	MBS-EP-A
	Langkaan I Talipapa	Calamba, Laguna	MBS-EP-B
	Langkaan I Talipapa	Unspecified	MBS-EP-C
Cabbage	FCIE Talipapa	San Pablo, Laguna	MBS-CAB-A
	Langkaan I Talipapa	Calamba, Laguna	MBS-CAB-B
Bitter gourd	FCIE Talipapa	San Pablo, Laguna	MBS-BTG-A
	Langkaan I Talipapa	Calamba, Laguna	MBS-BTG-B

FCIE- First Cavite Industrial Estate

Each vegetable sample was analyzed in duplicate and extracted in conjunction with fortified samples at LOQ and 10x LOQ to serve as the quality control checks. The residue of thiamethoxam and dinotefuran was below the limit of quantitation (0.01 mg/kg) for all samples. In contrast, one sample of eggplant was found to have imidacloprid residue above the set quantitation limit and is calculated to be 0.02 mg/kg. The results of the random market basket sampling, as well as the spiked quality controls, are summarized in **Table 8**.

In this study, a modified QuEChERS method based on internationally accepted and established guidelines from the International Union of Pure and Applied Chemistry, Codex Alimentarius Commission, and the EU-Directorate-General for Health and Food Safety (SANTE) was validated. The validation guidelines used for assessing the performance of analytical methods from single-laboratory measurements included parameters such as selectivity, specificity, linearity, accuracy(recovery), trueness (precision), and matrix effects. The representative commodities used encompass commodities with a high volume of production and overall consumption and production, as well as their potential for food safety risks. The analytes quantified were identified as substances of emerging concern, not only from an environmental aspect but also from a food safety aspect.

Table 8. Summary of the residue analysis results for neonicotinoids.

Laboratory Code	Dinotefuran, mg/kg	Imidacloprid, mg/kg	Thiamethoxam, mg/kg
MBS-EP-A1	< 0.01	< 0.01	< 0.01
MBS-EP-A2	< 0.01	< 0.01	< 0.01
MBS-EP-B1	< 0.01	< 0.01	< 0.01
MBS-EP-B2	< 0.01	< 0.01	< 0.01
MBS-EP-C1	< 0.01	0.02*	< 0.01
MBS-EP-C2	< 0.01	0.02*	< 0.01
EP-1x	0.01	0.01	0.01
EP-10x	0.1	0.1	0.1
MBS-CAB-A1	< 0.01	< 0.01	< 0.01
MBS- CAB -A2	< 0.01	< 0.01	< 0.01
MBS- CAB -B1	< 0.01	< 0.01	< 0.01
MBS- CAB -B2	< 0.01	< 0.01	< 0.01
CAB-1X	0.01 (100%)	0.01 (100%)	0.01 (100%)
CAB-10x	0.1 (100%)	0.1 (100%)	0.1 (100%)
MBS-BTG-A1	< 0.01	< 0.01	< 0.01
MBS- BTG -A2	< 0.01	< 0.01	< 0.01
MBS- BTG -B1	< 0.01	< 0.01	< 0.01
MBS- BTG -B2	< 0.01	< 0.01	< 0.01
BTG-1X	0.1 (100%)	0.1 (100%)	0.1 (100%)
BTG-10x	0.1 (100%)	0.1 (100%)	0.1 (100%)

*positive identification and quantification above LOQ

The selected methods were validated following the international guidelines and were found to meet the performance characteristics required for pesticide residue analysis methods. The trueness of the methods was within the range of 70-120 %, and the precision expressed in percent relative standard deviation was less than 20 %. The targeted limit of quantification of 0.01 mg/kg for all matrix-analyte combinations was satisfactorily met, ensuring reliable

quantification of pesticide residues. The established quantification limits of 0.01 mg/kg were below or at the existing national MRL /default maximum residue limits set by international regulatory authorities, indicating that the method is suitable for routine pesticide monitoring and compliance testing. Application of the method to real-world samples was demonstrated by analyzing market basket samples from the vicinity of Dasmariñas City, Cavite. Dinotefuran and thiamethoxam concentrations were less than the LOQ in all vegetable samples, while an incurred residue of imidacloprid was found in one of the eggplant samples above the LOQ, calculated at 0.02 mg/kg.

SUMMARY AND CONCLUSION

In summary, pesticide residue analysis is essential in the Pest Management Process. Pesticide analysis delivers vital information on pesticide residues that persist in the crop before it is marketed and consumed. Establishing pesticide concentrations enables informed choices about pesticide application, ultimately reducing its usage while ensuring effective pest management and safeguarding environmental and human health. This research offers an in-depth assessment of the suitability of the QuEChERS method for pesticide analysis, showcasing its performance in line with globally recognized validation standards. Validation broadens the range of matrix-analyte pairs that residue chemists can utilize to detect neonicotinoids at low levels quickly, easily, efficiently, and in an eco-friendly way, thus enhancing food safety and adherence to regulations, which in turn aids in safeguarding public health and the environment.

ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my research supervisors, Ms. Edna Mijares, Dr. Lilibeth Coo, and Dr. Pythias Espino, for providing invaluable guidance and insights throughout this research, to the staff of JEFCOR Laboratories, Inc. particularly, Ms. Loida Pacaro, Ms. Joselette Reyes, and Ms. Emyflor Guarino for their support during the study.

My sincerest thanks to the Crop Protection Association of the Philippines, for the financial support they have provided, LEADS Agricultural Products Corporation, and Mr. Aristeo Filipino†. Thank you to the Integrated Chemists of the Philippines for their support, and to the Bureau of Agriculture and Fisheries

Standards, Fertilizer and Pesticide Authority, the National Pesticide Analytical Laboratory, and the Organic Certification Center of the Philippines on practical information throughout the study.

To the Filipino farmer, whose daily sacrifice leads to national development. May this minute contribution thrust forward the agriculture and food safety of the country. My sincerest thanks to all who made this study possible.

REFERENCES CITED

ANASTASSIADES M, LEHOTAY SJ, STAJNBAHER D, SCHENCK FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International, 86(2), 412–431.

ANASTASSIADES M, SCHERBAUM E, TAŞDELEN, B. ŠTAJNBAHER, D. 2007. Recent Developments in QuEChERS Methodology for Pesticide Multiresidue Analysis. In Pesticide Chemistry (eds H. Ohkawa, H. Miyagawa and P.W. Lee). Retrieved from: https://doi.org/10.1002/9783527611249.ch46

AOAC Official Method 2007.01. 2007. Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.

CHEN M, TAO L, MCLEAN J, LU C. 2014. Quantitative analysis of neonicotinoid insecticide residues in foods: implication for dietary exposures. Journal of agricultural and food chemistry, 62(26), 6082–6090. Retrieved from: https://doi.org/10.1021/jf501397m.

CIMINO AM, BOYLES AL, THAYER KA, PERRY MJ. 2017. Effects of neonicotinoid pesticide exposure on human health: A systematic review. Environmental Health Perspectives, 125(2), 155–162. Retrieved from: https://doi.org/10.1289/EHP515

CODEX Alimentarius Commission (2017). Guidelines on Good Laboratory Practice in Pesticide Residue Analysis CAC/GL 40-1993 (Revision 2003. Amendment 2017).

CODEX Alimentarius Commission (2010). CAC/GL 41-1993: Portion of Commodities to Which Maximum Residue Limits Apply, and Which is Analyzed. Amended 2010

CODEX Alimentarius Commission (1999). CAC/GL 33-1999: Recommended Methods of Sampling for the Determination of Pesticide Residues for Compliance with MRLs.

CODEX Alimentarius Commission (2005). CAC/GL 56-2005 Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation, and Quantitative Determination of Residues.

CODEX Alimentarius Commission (2017). CXG 90-2017: Guidelines on Performance Criteria for Method of Analysis for the Determination of Pesticide Residues in Food and Feed.

CODEX Alimentarius Commission (2010). CAC/GL 40-1993: Guidelines on Good Laboratory Practice in Pesticide Residue Analysis. Revision 2003. Amendment 2010.

JESCHKE, P. NAUEN R, SCHINDLEMR, ELBERT A. 2011. Overview of the status and global strategy for neonicotinoids', Journal of Agricultural and Food Chemistry, 59(7), pp. 2897–2908. doi:10.1021/jf101303g.

THOMPSON M, ELLISON SLR, WOOD R. 2002. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report), Pure and Applied Chemistry. Retrieved from: https://publications.iupac.org/pac/74/5/0835/index.html

SANTE/12682/2019 – Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

SANCO/10684/2009: Method validation and quality control procedures for pesticide residue analysis in food and feed. Retrieved 2021 June

TOMLIN CD.S. 2006 The Pesticide Manual, A World Compendium, 14th Edition British Crop Protection Council. Alton, Hampshire, 186-187.