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Article

Optimization of an analytical method for carbaryl and chlorpyrifos residues determination by LC-MS/MS

Mohammad Dalower Hossain Prodhan[1]*, Marina Afroze[1], Afroza Begum[1], Md. Sultan Ahmed[1], Nirmal Kumar Dutta[1] and Debasish Sarker[2]

[1]Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2]Director General, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

*Corresponding author: Mohammad Dalower Hossain Prodhan, Pesticide Analytical Laboratory, Pesticide Research & Environmental Toxicology Section, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh. Phone: +8801819849657; E-mail: mdhprodhan@gmail.com

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Abstract: The present study was aimed to optimize an analytical method for the quantification of carbamate and organophosphorus insecticide residues using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). A series of experiments were conducted to select the parent ion and precursor ion and based on these findings, the analytical method for the determination of carbamate and organophosphorus insecticide residues using LC-MS/MS was developed. The electrospray ionization (ESI) mode was used to develop the method. The linearity of the developed analytical method was very good and it was 0.999 for carbaryl, while it was 0.998 for chlorpyrifos. The optimization of MS/MS parameters has been done properly for both of the selected pesticides through direct infusion of 100 ug/L pure analytical standard solutions.

Keywords: carbamate insecticide residue; organophosphorus insecticide residue; LC-MS/MS; ESI mode

1. Introduction

In modern agricultural practices pesticides are commonly used to protect crops from being destroyed by pest and plant diseases and thus ensure abundant supply of quality foods. At present, the farmers in all over the world are using different neonicotinoid insecticides along with some other pesticides which have less residual effects, however in the developing world, the farmers are still using different carbamates and organophosphorus pesticides like carbaryl, chlorpyrifos, dimethote, fenitrothion etc. (Prodhan *et al*., 2022). In Bangladesh, the farmers are also using these carbamates and organophosphorus pesticides which have long residual effects in the environment. Thus, it is necessary to develop the analytical method using the modern analytical technique to detect and quantify the residues at trace levels.

Till today, High Performance Liquid Chromatography (HPLC), Liquid Chromatography associated with Mass Spectrometry (LC-MS), Gas Chromatography (GC), and Gas Chromatography associated with Mass Spectrometry (GC-MS) are the most commonly used analytical techniques. Among these analytical methods, Gas Chromatography (GC) was used for the determination of pesticide residues in a variety of matrices such as fruits and vegetables (Tasnim *et al*., 2022; Kabir *et al*., 2007; 2008; 2008a; Hoque *et al*., 2021; Habib *et al*., 2021; Prodhan *et al*., 2021; 2021a; 2021b; 2018; 2018a; 2018b; 2010; 2009; Rahman *et al*., 2021; Nahar *et al*.,

2020; Islam *et al*., 2021; 2019; 2019a; Hossain *et al*., 2014; Alam *et al*., 2022; Parvin *et al*., 2021; Parven *et al*., 2021; Hasan *et al*., 2021; Islam *et al*., 2014). Since it is a great approach that typically minimizes the superfluous cleaning stages, presents minimal probability of false-positive findings, and reduces the analysis time and cost, LC-MS/MS has been utilized for the identification of pesticide residues in the extracts of fruits and vegetables (Prodhan *et al*., 2022; Heimstra and Kok, 2007).

Presently, among all the analytical techniques, the Liquid Chromatography tandem Mass Spectrometry was used widely as it is a powerful and well accepted analytical technique (Prodhan *et al*., 2022). The time of flight mass spectrometry, single quadrupole, triple quadrupole, ion trap, and other mass analyzers are some of the mass analyzers utilized in LC-MS. To assess various pesticide residues from a range of matrices, LC-MS/MS with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) source is frequently utilized (Jansson *et al*., 2004; Fan *et al*., 2014; Prodhan *et al*., 2018c; 2016; 2016a; 2015; 2015a; Obana *et al*., 2003). Only a small number of pesticides can be examined using both GC-MS and LC-MS methods. However, LC-MS was thought to have a broader use than GC-MS (Mol *et al*., 2008). The capability of identifying pesticides with various chemical structures in food at concentrations equivalent to those obtained by GC-MS has increased the capability to LC-MS/MS with ESI and APCI source (Pico *et al*., 2006). With this view, this study was initiated to develop an analytical method for the quantification of carbaryl and chlorpyrifos residues using Liquid Chromatography tandem Mass spectrometry (LC-MS/MS).

2. Materials and Methods

2.1. Chemicals and reagents

Carbaryl and chlorpyrifos Certified Reference Materials (CRM) was purchased from Sigma-Aldrich (St. Louis, MO, USA) through SAF Scientific, Bangladesh Limited. Purchased from Merck were the acetonitrile (HPLC grade), methanol (MS grade), and water (MS grade) (Darmstadt, Germany). Anhydrous magnesium sulphate (MgSO4), primary secondary amine (PSA), and sodium chloride were all bought from Chem-Lab in Zedelgem, Belgium, through Asa Scientific Ltd. in Dhaka, Bangladesh. Through SAF Scientific, Bangladesh Limited, formic acid, ammonium acetate, and ammonium formate of mass spectrometry quality were bought from Fluka (Buchs, Switzerland).

2.2. Preparation of pesticide standard solution

Carbaryl and chlorpyrifos were produced separately as pesticide standard stock solutions in methanol at a concentration of 1000 mg/L and kept at -200C until use. By mixing the required volume of each individual stock solution in a volumetric flask (50 ml) and bringing the mixture to volume with the appropriate solvent, a mixed standard solution of 50 mg/L in methanol containing all the aforementioned pesticides was created. The standard solution of 50 mg/L was then converted into an intermediate standard solution of 10 mg/L in a suitable solvent. Then, ten separate 10-ml volumetric working standard solutions of 0.01, 0.05, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mg/L in methanol were created by adding the necessary quantity from a 10 mg/L intermediate mixed standard solution.

2.3. Instrumental analysis

Thermo Scientific TSQ Quantum Access Max triple quadrupole mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA) linked to a UHPLC- DIONEX Ultimate 3000 was used to create the analytical procedure. The Heated Electrospray Ionization (HESI) source-equipped with tandem mass spectrometer ran in positive ion mode. To collect and process the data, the Xcalibur Data System was employed. Carbaryl and chlorpyrifos were determined using two distinct procedures. The determination of the analytes was achieved by a Hypersil Gold C18 column (2.5 μm, 50 × 2.1 mm i.d).

The LC pump gradient program was: 0-5 min, 20% mobile phase B; 5-16 min, 100% mobile phase B; 16-27.5 min, 20% mobile phase B. The mobile phase flow rate was 0-18.5 min: 0.2 mL/min; 18.5-25.5 min: 0.5 mL/min and 25.5-27.5 min: 0.2 mL/min. In this investigation, two mobility phases were employed. The mobile phase B was a 10:90 mixture of water and methanol with 5 mM ammonium acetate whereas the mobile phase A was a 90:10 mixture of water and methanol with 5 mM ammonium acetate. For the chromatographic separation, a HyPurity C18 analytical column (50 mm X 2.1 mm i.d., 3 m particle size) was employed (Thermo Scientific). The column oven's temperature was 40^0C, and the injection volume was 20 L. The total run time was 27.50 min.

2.4. Optimization of mass spectrometry operating conditions

Using a standard solution of 100 ug/L in LC-MS grade Methanol, direct infusion was utilized to acquire the mass spectra, optimal collision energy, and tube lens values for the chosen pesticides. To optimize these values

for each pesticide, a number of tests were carried out in positive mode and with various solvents. By using flow injections of the chosen mobile phase, the ion source's operational parameters (spray voltage, sheath gas, auxiliary gas, capillary temperature, and skimmer offset) were each individually tuned for each pesticide (MeOH and LC-MS grade water).

3. Results and Discussion

3.1. Method development and LC–MS/MS parameters optimization

To extract the precursor and product ions, each insecticide was directly infused (standard solution, 100 ug/L in MeOH). Breakdown curves were also produced when collision energy (CE) and tube lens voltage (TL) were each individually tuned for each analyte. Figure 1 and 2 displays the mass spectra and fragments of carbaryl and chlorpyrifos, respectively.

In this study, the precursor ion of carbaryl was [M+H]+ (m/z 202.09) and the quantifier (Q) and qualifier (q) ions were m/z 127.1 and m/z 145.08, respectively, being in agreement with Prodhan *et al*. (2015). In case of chlorpyrifos, the precursor ion was [M+H]+ m/z 349.71; the quantifier ion was m/z 96.6 and the qualifier ion was m/z 170.9. Each pesticide's specific mobile phase composition has been carefully adjusted. Different mobile phases containing water and various additions, including formic acid, ammonium acetate, and ammonium formate at varying concentrations, were examined. The organic phase was either methanol or acetonitrile. In order to get the greatest peak area and best signal-to-noise ratio, ultrapure water and MeOH were mixed in various ratios. Both analytes underwent isocratic elution. To effectively clear the column from the non-polar matrix interferences after their elution, the organic mobile phase was raised to 100%.

The chromatograms of the carbaryl and chlorpyrifos are illustrated in Figure 3 and 4. The operating conditions of ESI were as follows: Sheath gas (nitrogen) pressure was 30 arbitrary units; Auxiliary gas (nitrogen) pressure was 10 arbitrary units; Spray voltage was 4000 V; Capillary temperature was 325ºC. The collision gas pressure was 1.5 mTorr. In the Selected Reaction Monitoring (SRM) mode, the acquisition was made. Table 1 lists the parent, quantification, and confirmation ions.

3.2. Preparation of calibration curve

The standard solutions of carbaryl and chlorpyrifos having different concentrations were produced and injected with the appropriate instrument settings. The samples were calibrated (in terms of retention time, peak area, etc.) using a five-point calibration curve of the relevant pesticide's reference solution. The retention time of each peak served as its identifier. For each of the chosen pesticides, the calibration curves created with various concentrations are shown in Figures 5 and 6. The calibration concentrations for carbaryl varied from 1 ug/L to100 ug/L, whereas those for chlorpyrifos ranged from 1 ug/L to 200 ug/L. Both pesticides' calibration curves had extremely high linearity, and their coefficient of determination was ≥0.998.

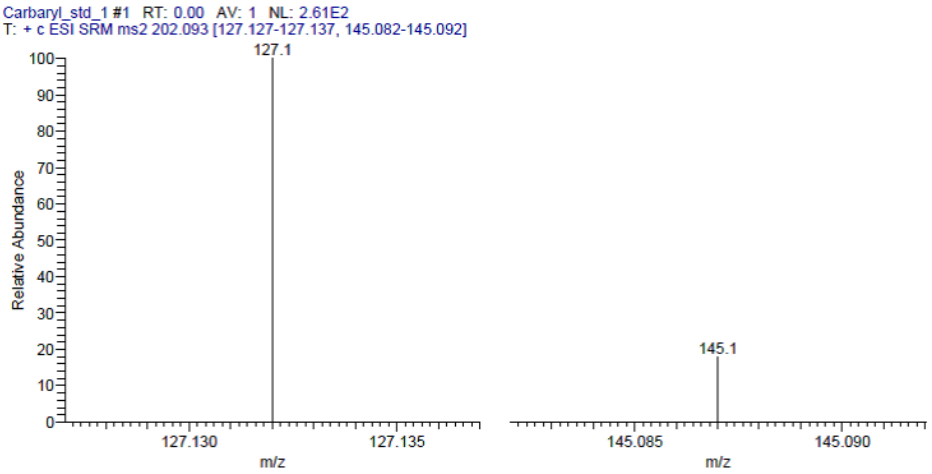

Figure 1. MS-MS spectra of carbaryl.

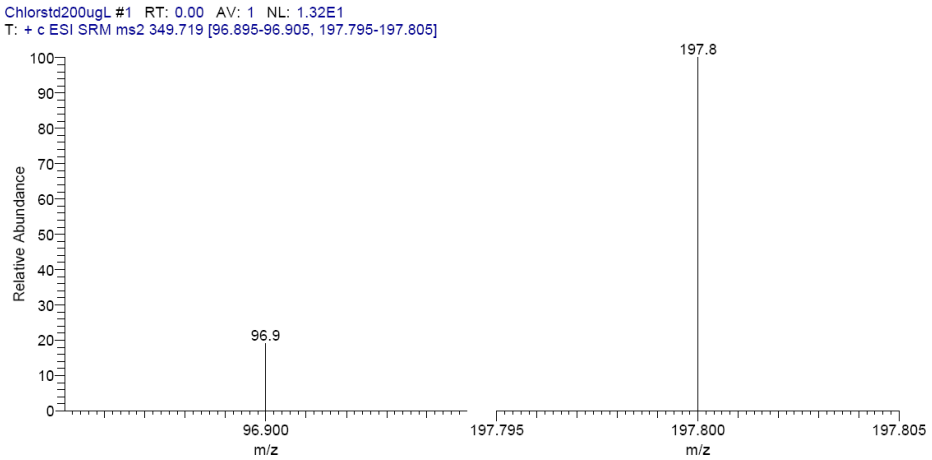

Figure 2. MS-MS spectra of chlorpyrifos.

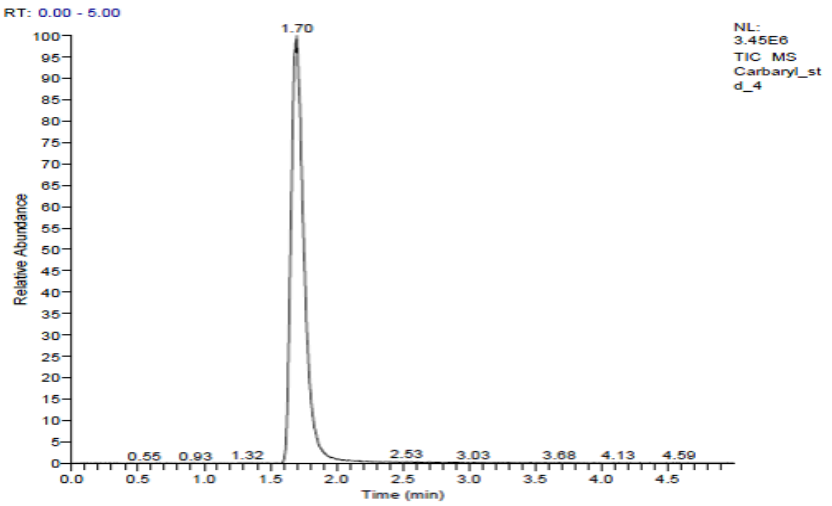

Figure 3. Chromatogram of carbaryl showing retention time.

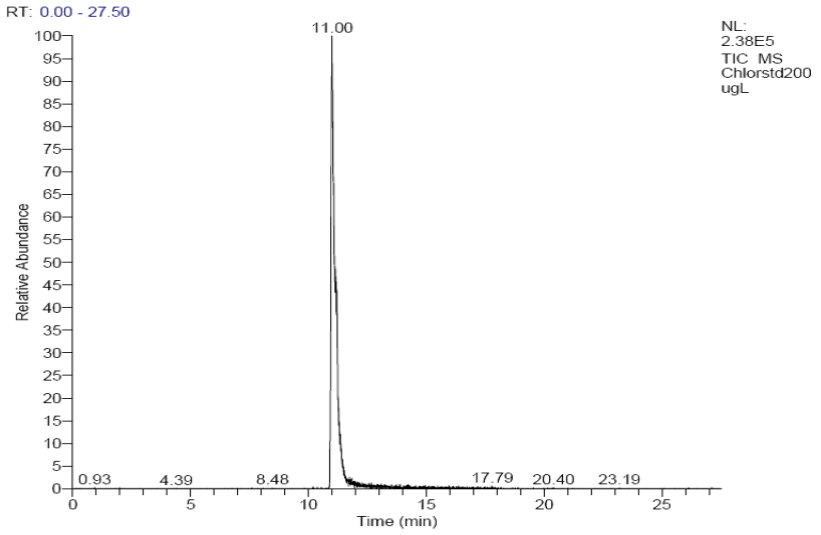

Figure 4. Chromatogram of chlorpyrifos showing retention time.

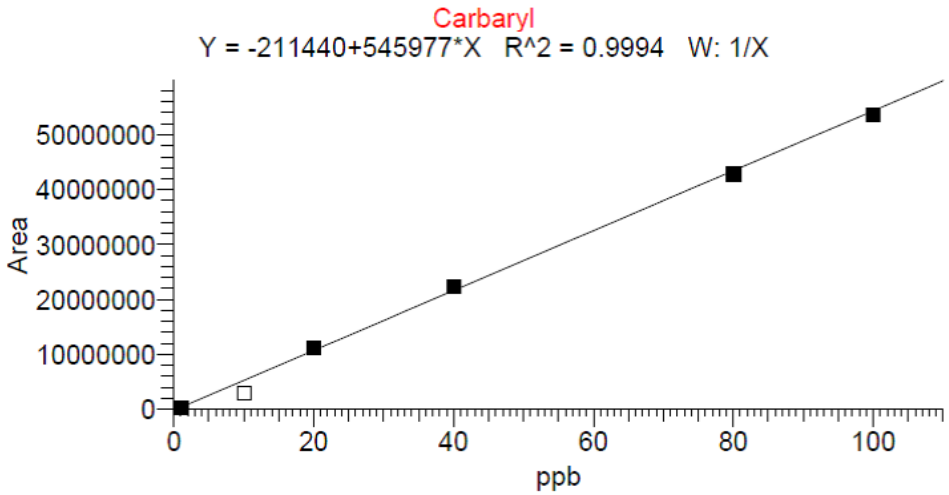

Figure 5. Calibration curve of carbaryl made with different concentrations ranging from 1 ug/L to 100 ug/L.

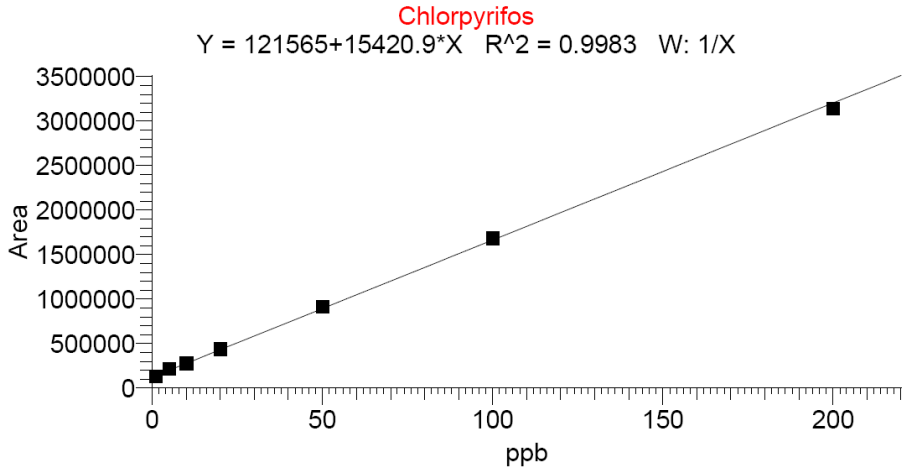

Figure 6. Calibration curve of chlorpyrifos made with different concentrations ranging from 1 ug/L to 200 ug/L.

Table 1. LC-MS/MS parameters for the quantification of carbaryl and chlorpyrifos residues.

Pesticides	RT (min.)	Parent ion	Quantification ion (m/z)	CE (V)	Confirmation ion (m/z)	CE (V)
Carbaryl	1.70	202.09	127.1	25	145.08	6
Chlorpyrifos	11.00	349.72	96.9	31	197.8	18

4. Conclusions

A precise analytical method was developed for quantifying carbaryl and chlorpyrifos residues using LC-MS/MS. The linearity of the developed analytical method was very good and it was ≥ 0.998. The developed method can be used successfully for the quantification of carbaryl and chlorpyrifos residues in food and the environmental samples as well. The results of this investigation will assist the analysts in appropriately analyzing the chosen pesticide residues.

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Data availability
All relevant data are within the manuscript.

Conflict of interest
None to declare.

Authors' contribution
Conceptualization: [Mohammad Dalower Hossain Prodhan]; Methodology: [Mohammad Dalower Hossain Prodhan]; Formal analysis and investigation: [Mohammad Dalower Hossain Prodhan, Marina Afroze, Afroza Begum]; Writing - original draft preparation: [Mohammad Dalower Hossain Prodhan]; Writing - review and editing: [Mohammad Dalower Hossain Prodhan, Md. Sultan Ahmed, Nirmal Kumar Dutta and Debasish Sarker]. All authors have read and approved the manuscript.

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