Article

Development of analytical method for pesticide residue determination using LC-MS/MS

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Abstract: This study was initiated to develop an accurate analytical method for the determination of neonicotinoid and organophosphorus insecticide using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). Several experiments were conducted to select the parent ion and precursor ion and based on this outcome, the analytical method for the determination of thiamethoxam and dimethoate using LC-MS/MS was developed. The heated electrospray ionization (HESI) mode was used to develop the method. The linearity of the developed analytical method was very good and it was 0.998 for both of the selected pesticides. The optimization of MS/MS parameters has been done properly for both of the selected pesticides through direct infusion of 100 ug/L standard solutions.

1. Introduction

To determine pesticide residues in fruits, vegetables and other matrices Gas Chromatography (GC), Gas Chromatography associated with Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography associated with Mass Spectrometry (LC-MS) are the most commonly used techniques. Among these analytical techniques, GC was used for the determination of pesticide residues in a wide variety of matrices including fruits and vegetables (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). In recent years, LC-MS/MS has been used for the determination of pesticide residues in the extracts of fruits and vegetables as it is an excellent technique which generally reduces the excessive cleanup steps, exhibits little chance of false-positive findings, and reduces the analysis time and cost (Hiemstra M. and Kok A de. 2007).

Liquid Chromatography Mass Spectrometry is a powerful and well accepted analytical technique that has very good sensitivity. Different mass analyzers are used in LC/MS, including single quadrupole, triple quadrupole, ion trap, time of flight mass spectrometry (TOF-MS). LC-MS/MS with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) source are used widely to analyze multiple pesticide residues

Asian Australas. J. Food Saf. Secur. 2022, 6 (2)

66

from a wide variety of matrices (Caboni *et al.,* 2008; Jansson C. *et al.,* 2004; Fan *et al.,* 2014; Prodhan *et al.,* 2018c; 2016; 2016a; 2015; 2015a; Obana *et al.,* 2003). A very few pesticides can be analyzed by both GC-MS and LC-MS techniques. But, LC-MS was considered to cover a wider scope than GC-MS (Mol HGJ *et al.,* 2008). LC-MS/MS with ESI and APCI source have improved the feasibility of the identification of pesticides of different chemical structures in food at concentrations comparable to those obtained by GC-MS (Pico *et al.,* 2006). Therefore, the present study was initiated to develop analytical method for the determination of thiamethoxam and dimethoate using Liquid Chromatography Triple Quadrupole Mass spectrometry (LC-MS/MS).

2. Materials and Methods
2.1. Chemicals and reagents
Reference standards of dimethoate and thiamethoxam were obtained from Sigma-Aldrich (St Louis, MO, USA) via SAF scientific, Bangladesh Limited. The methanol (MS grade), acetonitrile (HPLC grade), and water (MS grade) were purchased from Merck (Darmstadt, Germany). Sodium chloride was purchased from Chem-Lab (Zedelgem, Belgium), anhydrous magnesium sulphate ($MgSO_4$) and Primary Secondary Amine (PSA) was from Agilent (Santa Clara, CA, USA) via Asa Scientific Ltd., Dhaka, Bangladesh. Formic acid, ammonium acetate and ammonium formate of mass spectrometry grade was purchased from Fluka (Buchs, Switzerland) via SAF scientific, Bangladesh Limited.

2.2. Preparation of pesticide standard solution
Pesticide standard stock solutions of dimethoate and thiamethoxam were prepared separately in methanol at a concentration of 1000 mg/L and stored at -20^0C until use. A mixed standard solution of 50 mg/L in methanol containing all the aforementioned pesticides was prepared by adding the appropriate volume of each individual stock solution in a volumetric flask (50 ml) and made to volume by addition of appropriate solvent. Afterwards, an intermediate standard solution of 10 mg/L in suitable solvent was prepared from the standard solution of 50 mg/L. Then 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 prepared by transferring the appropriate amount from 10 mg/L intermediate mixed standard solution into ten separate 10-ml volumetric flasks.

2.3. Instrumental analysis
The development of analytical method was accomplished using a Thermo Scientific TSQ Quantum Access Max triple quadrupole mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA) connected to a UHPLC- DIONEX Ultimate 3000. The triple quadrupole mass spectrometer which was equipped with a Heated Electrospray Ionization (HESI) source operated at the positive ion mode. The Xcalibur Data System was used to the acquisition and processing of data. Two individual and specific methods were developed for the determination of thiamethoxam and dimethoate. 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. There are two mobile phase was used in this study. The mobile phase A consisted of a 90:10, water: methanol mixture containing 5 mM of ammonium acetate, however the mobile phase B was a 10:90, water: methanol mixture containing 5 mM of ammonium acetate. A HyPurity C18 analytical column (50 mm X 2.1 mm i.d., 3 μm particle size) was used for the chromatographic separation (Thermo Scientific). The injection volume was 20 μL and the column oven temperature was 40^0C. The total run time was 27.50 min.

2.4. Optimization of mass spectrometry operating conditions
Direct infusion was used to obtain the mass spectra and the optimum collision energy and tube lens values for each pesticide using a standard solution of 100 ug/L in LC-MS grade Methanol. Several experiments in positive mode and in different solvents were performed to optimize these parameters for each pesticide. The operating parameters of the ion source (spray voltage, sheath gas, auxiliary gas, capillary temperature and skimmer offset) were optimized separately for each pesticide by flow injections of the selected mobile phase (MeOH and LC-MS grade water).

3. Results and Discussion
3.1. Method development and LC–MS/MS parameters optimization
Direct infusion of each pesticide (standard solution, 100 ug/L in MeOH) was accomplished to obtain the precursor and product ions. Collision energy (CE) and tube lens voltage (TL) were optimized for each analyte separately and breakdown curves were also obtained. The mass spectra and the fragments of thiamethoxam and dimethoate are shown in Figure 1 and Figure 2.

In this study, the precursor ion of thiamethoxam was [M+H]+ (m/z 291.9) and the quantifier (Q) and qualifier (q) ions were m/z 211.0 and m/z 181.0, respectively, being in agreement with Prodhan et al., 2015. For dimethoate, the precursor ion was [M+H]+ m/z 229.9; the quantifier ion was m/z 125.0 and the qualifier ion was m/z 170.9. The constitution of the mobile phases has been thoroughly optimized for each pesticide separately. Different mobile phases consisting of methanol or acetonitrile as the organic phase and water with different additives, such as formic acid, ammonium acetate and ammonium formate at various concentrations were tested. Ultrapure water and MeOH, in different ratios were chosen, as they proved to be optimum in terms of highest peak area and signal-to-noise ratio. Both analytes were eluted in isocratic conditions. After their elution, the organic mobile phase was increased to 100% in order to achieve efficient clean-up of the column from the non-polar matrix interferences.

The chromatograms of the both pesticides are illustrated in Figure 3 and Figure 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 325^0C. The collision gas pressure was 1.5 mTorr. The acquisition was made in the Selected Reaction Monitoring (SRM) mode. The parent ion, quantification and confirmation ions are presented in Table 1.

3.2. Preparation of calibration curve
The standard solutions of different concentrations of each pesticide were prepared and injected with suitable instrument parameters. The samples were calibrated (retention time, peak area, etc.) against five pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time. The calibration curves prepared with different concentrations are presented in Figures 5 and 6 for both of the selected pesticides. In case of thiamethoxam, the calibration concentrations ranged from 1 ug/L to 200 ug/L, while it was 10 ug/L to 50 ug/L for dimethoate. The linearity of the calibration curve for both of the pesticides was very good and the co-efficient of determination was 0.998.
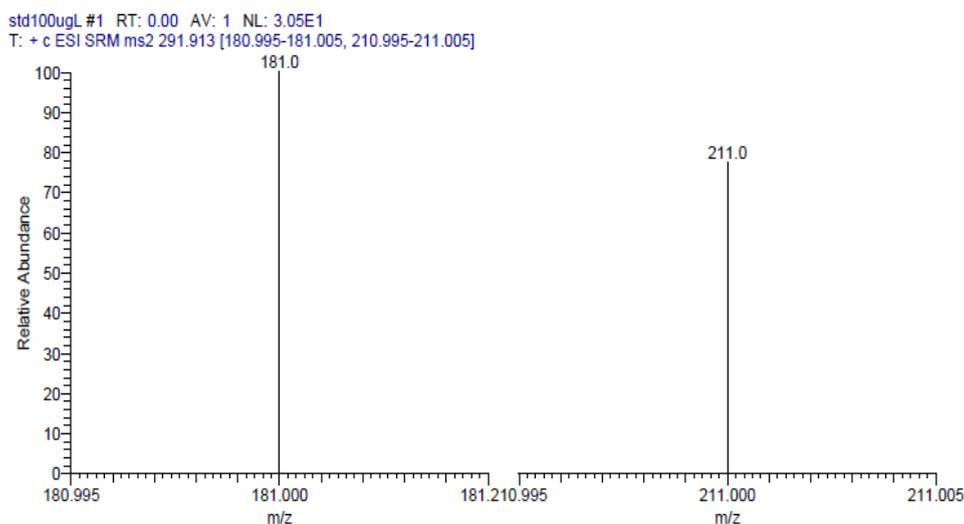

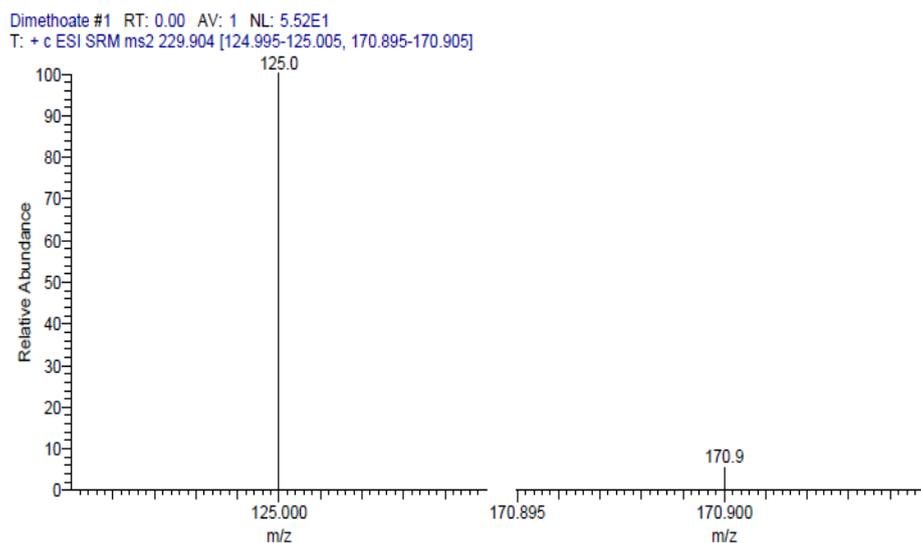**Figure 1. MS-MS Spectra of thiamethoxam.**

Asian Australas. J. Food Saf. Secur. 2022, 6 (2)

68

Figure 2. MS-MS Spectra of dimethoate.

Table 1. LC-MS/MS parameters for the selected pesticides.

Pesticides	RT (min.)	Parent ion	Quantification ion (m/z)	CE (V)	Confirmation ion (m/z)	CE (V)
Thiamethoxam	2.44	291.9	211.0	24	181.0	112
Dimethoate	4.11	229.9	125.0	19	170.9	14
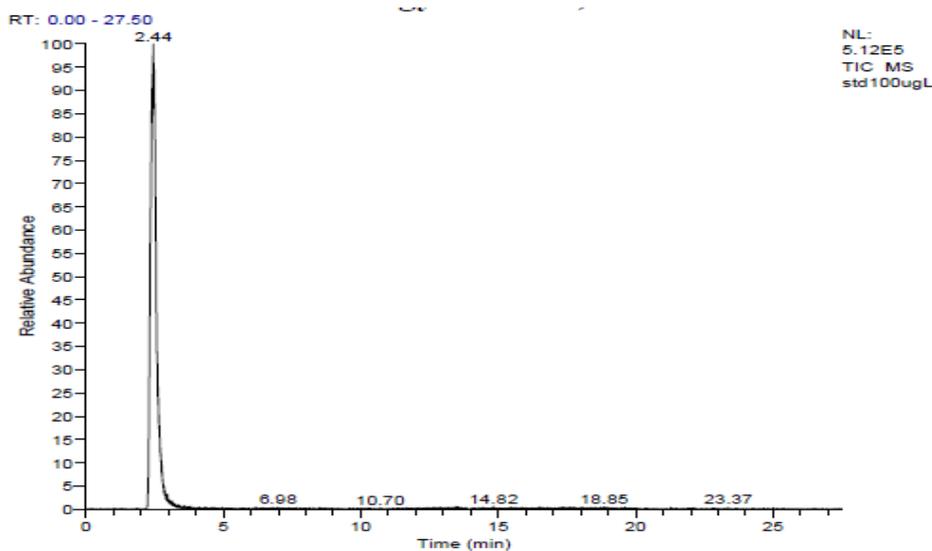
Figure 3. Chromatogram of thiamethoxam showing retention time.

Asian Australas. J. Food Saf. Secur. 2022, 6 (2)
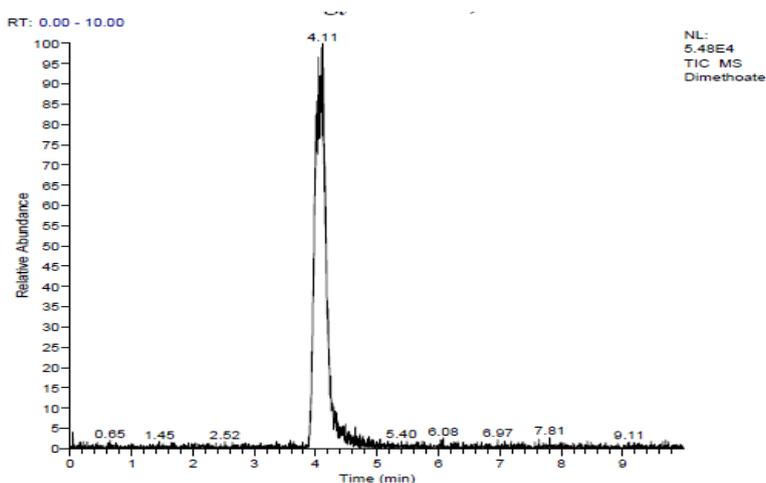
69

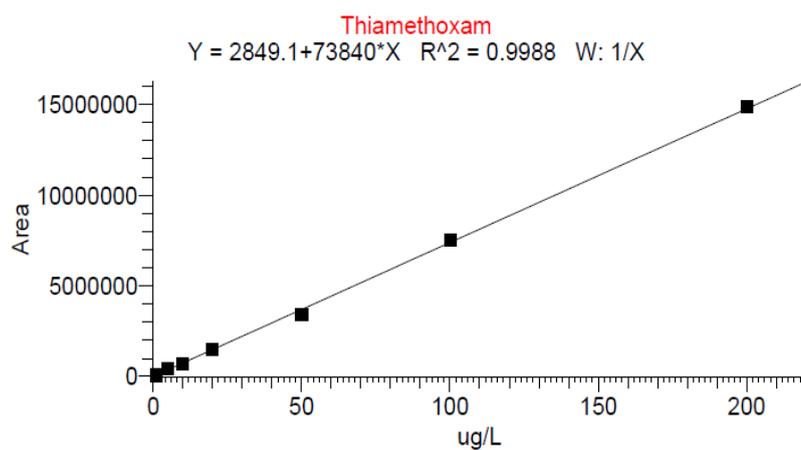**Figure 4. Chromatogram of dimethoate showing retention time.**

Figure 5. Calibration curve of thiamethoxam made with different concentrations ranging from 1 ug/L to 200 ug/L.
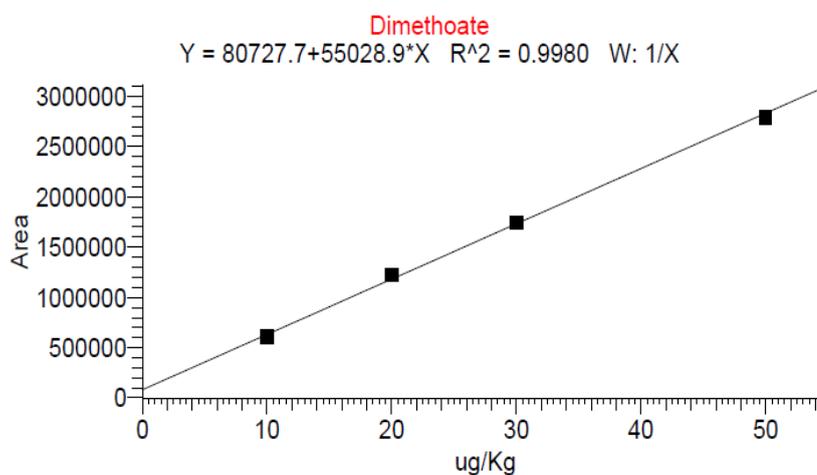

Figure 6. Calibration curve of dimethoate made with different concentrations ranging from 10 ug/L to 50 ug/L.

Asian Australas. J. Food Saf. Secur. 2022, 6 (2)

70

4. Conclusions

In this study, an efficient analytical method was developed for the determination of thiamethoxam and dimethoate using LC-MS/MS. The linearity of the developed analytical method was very good and it was 0.998. The proposed method can be used for the analysis of the selected pesticides in fruits and vegetables. The findings of this study will help the scientist/ analysts to quantify the selected pesticide residues accurately.

Data availability

Data are contained within the article.

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, Afroza Begum, Marina Afroze]; 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 final manuscript.

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Asian Australas. J. Food Saf. Secur. 2022, 6 (2)

71

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Asian Australas. J. Food Saf. Secur. 2022, 6 (2)

72

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