Vol. 8, No. 3, 2017
ISSN 2233-4203/ e-ISSN 2093-8950
INVITED ARTICLE www.msletters.org | Mass Spectrometry Letters

Inspection of the Fragmentation Pathway for Thiamethoxam

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Received August 03, 2017; Accepted September 20, 2017
First published on the web September 30, 2017; DOI: 10.5478/MSL.2017.8.3.65

Abstract : Thiamethoxam is one of the main suspect in honeybee colony collapse disorder (CCD). Due to this reason, thiamethoxam including imidacloprid and clothianidin has been banned for two years in some Europe countries. The CCD phenomenon has also been reported in Korea. Regarding this issue and needs, a new project has started to develop the method for the quatitation of thiamethoxam using isotope dilution mass spectrometry (IDMS). In the process of optimization for the IDMS method with thiamethoxam and thiamethoxam-d_3, we observed that the fragment peaks did not correspond to the fragmentation pathway as published elsewhere. Here, we proposed a candidate fragmentation pathway. To validate the proposed fragmentation pathway, another isotope analogue, thiamethoxam-d_4, was introduced and the MS/MS spectra of both isotope analogues were compared. In addition, the MS/MS/MS spectra of thiamethoxam were inspected for more evidence of the candidate pathway. Those spectra indicated that the proposed fragmentation pathway could be used to assign the fragment peaks of thiamethoxam.

Keywords : neonicotinoid insecticide, thiamethoxam, thiamethoxam-d_3, thiamethoxam-d_4, fragmentation pathway, isotope dilution mass spectrometry

Introduction

Thiamethoxam is a second-generation neonicotinoid insecticide and belongs to a neuro-active insecticide class.[1] They are the most extensively used insecticides because they are less toxic to humans but have high activity against pests and insects.[2-3] However, these neonicotinoid insecticides are a main suspect of honeybee colony collapse disorder (CCD),[4-7] and several countries in Europe had banned the use of the neonicotinoid insecticides, thiamethoxam, imidacloprid, and chlothianidin, for two years (2013-2015) to monitor a change (Two-year European moratorium).[8-9] Two-year European moratorium remains under review since 2015. In Korea, these neonicotinoid insecticides have also been used widely and the CCD phenomenon has been reported.[10-12] Following the two-year moratorium, the Korean government regulated the use of thiamethoxam, imidacloprid, and chlothianidin for a limited time during flowering season. In addition, the new registration and changes for three pesticides has been prohibited considering a two-year moratorium by the government.

Our laboratory has developed and dissemenated certified reference materials (CRMs) for the analysis of residue pesticides including organophosphorus, organochloride, and carbamate pesticides.[13-14] A new project has started to develop the analytical method and provide CRMs for the analysis of thiamethoxam, imidacloprid, and chlothianidin. Our laboratory has employed isotope dilution mass spectrometry (IDMS) as a reference method for accurate determination of analytes and for the value assignment of those analytes in CRMs. To develop IDMS methods, conditions of mass spectrometry (MS) and tandem mass spectrometry (MS/MS) have to be optimized.

During the optimization of thiamethoxam and thiamethoxam isotope analogues, we observed that peak assignment of daughter ions with a fragmentation pathway published in other literatures[15-17] did not correspond to the MS fragment peaks obtained in our work. Thus, to define the fragmentation pathway and assign the daughter ion peaks in MS/MS spectrum, a candidate fragmentation pathway was proposed. To validate the proposed fragmentation pathway, the MS/MS spectra of two different isotope analogues of thiamethoxam, thiamethoxam-d_3 and thiamethoxam-d_4, were analyzed and inspected. In addition, MS/MS/MS spectra of thiamethoxam supported the candidate fragmentation pathway.

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Experimental

Materials

Thiamethoxam, thiamethoxam-d_3, thiamethoxam-d_4 were purchased from Dr. Ehrenstorfer (Germany), CDN Isotopes (Canada), and LGC Standards GmbH (Germany), respectively. Their chemical structures are illustrated in Scheme 1. HPLC grade acetonitrile was obtained from Burdick and Jackson (Muskegon, MI, USA). Pure water was prepared by passing through a Millipore Corp Milli-Q RG purification system.

Mass spectrometric analysis

To prepare stock solutions, 0.1 mg of thiamethoxam, thiamethoxam-d_3, or thiamethoxam-d_4, were dissolved in 40 g of acetonitrile and water mixture (50:50, v/v), respectively.

The MS/MS spectra of thiamethoxam, thiamethoxam-d_3, and thiamethoxam-d_4 were obtained with ThermoElectron (San Jose, CA) TSQ quantum mass spectrometer equipped with ESI. The ESI voltage was 3500 V and the capillary temperature was 350°C. The N_2 gas was used for nebulization gas and collision gas for collision induced dissociation (CID). The infusion flow rate was 5 μL/min and the collision energy of three compounds was 15 eV.

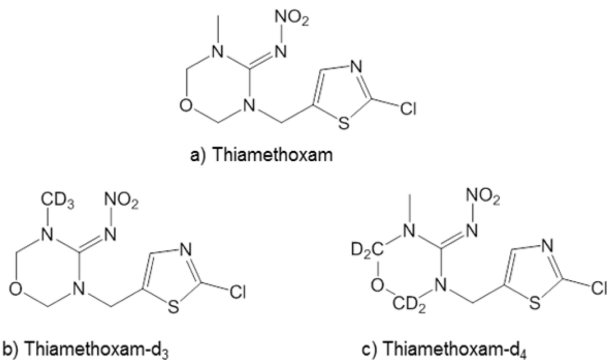
Results and Discussion

MS and MS/MS analysis

To develop the IDMS method, the conditions and parameters of MS and MS/MS for thiamethoxam were optimized as described in the experimental section. The MS/MS spectrum of thiamethoxam is shown in Figure 1 (a). There is a parent peak $[M+H]^+$ ion at *m/z* 292, the first daughter ion is a $[M\text{-}NO_2\text{-}Cl]^+$ ion at *m/z* 211 and the second daughter ion is at *m/z* 181 of thiamethoxam. According to the literature[15-17], the second daughter ion at *m/z* 181 was assigned as fragmentation by a loss of N_2O_2, Cl and CH_3. The fragmentation pattern was shown in the first column of Table 1. The first daughter ion at *m/z* 211 was selected for a quantitation channel and the second daughter ion at *m/z* 181 was selected for a confirmatory channel (for the validation of MS/MS quantitation). Thiamethoxam-d_3 was obtained for the corresponding isotope analogue. Similar to native thiamethoxam, the conditions and parameters of thiamethoxam-d_3 for MS and MS/MS were optimized. The MS/MS spectrum of thiamethoxam-d_3 is shown in Figure 1 (b). We expected two daughter ions, the $[M\text{-}NO_2\text{-}Cl]^+$ ion at *m/z* 214 and the $[M\text{-}N_2O_2\text{-}CH_3\text{-}Cl]^+$ ion at *m/z* 181. These correspond to the native thiamethoxam according to the literature. However, the spectrum showed no $[M\text{-}N_2O_2\text{-}CH_3\text{-}Cl]^+$ peak at *m/z* 181, but it did display a peak at *m/z* 184 (Figure 1. (b)).

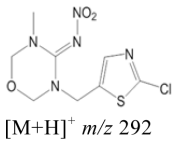
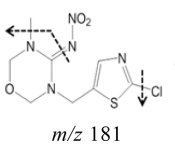
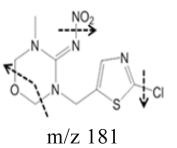
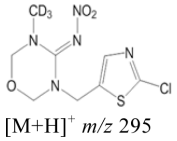
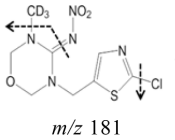
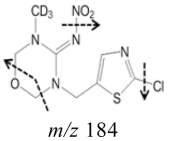
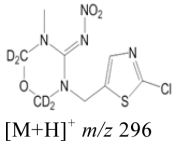
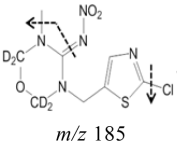
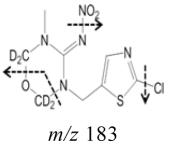
We again inspected the structure of thiamethoxam and tried to find another available pathway for the peak at *m/z* 184. Urzedo *et al.*[18] studied the photolytic degradation of thiamethoxam and proposed the fragment pathway. They suggested a loss of CH_2O from the 6-ring in thiamethoxam. However, they used UV radiated thiamethoxam, which did not exactly represent the fragmentation pathway of native thiamethoxam. Thus, we proposed a candidate pathway regarding the loss of CH_2O shown in the second column of Table 1. To validate the proposed pathway, we searched other isotope analogues such as a C_{13} substitute analogue or the other position deuterium analogue. Fortunately, there is another deuterium isotope analogue, thiamethoxam-d_4 that is commercially available.

Scheme 1 says thiamethoxam-d_3 has deuterium in a branch of the ring as CD_3 form, and thiamethoxam-d_4 has deuterium as two CD_2 forms in the 6-ring. Therefore, the peak at *m/z* 185 sould be produced if the pathway described in the literature is a proper assignment. However, we observed the peak at *m/z* 183 produced via the proposed pathway in this study. These peaks are summarized in the Table 1. The MS/MS results for thiamethoxam-d_4 included a parent ion at *m/*



Scheme 1. Chemical structures of thiamethoxam and its isotope analogues.

Table 1. Summary of daughter ions and fragmentation pathways for thiamethoxam and its isotope analogues.

	Published pathway	Proposed pathway	Measured value
			*m/z* 181
$[M+H]^+$ *m/z* 292	*m/z* 181	m/z 181	
			*m/z* 184
$[M+H]^+$ *m/z* 295	*m/z* 181	*m/z* 184	
			*m/z* 183
$[M+H]^+$ *m/z* 296	*m/z* 185	*m/z* 183	

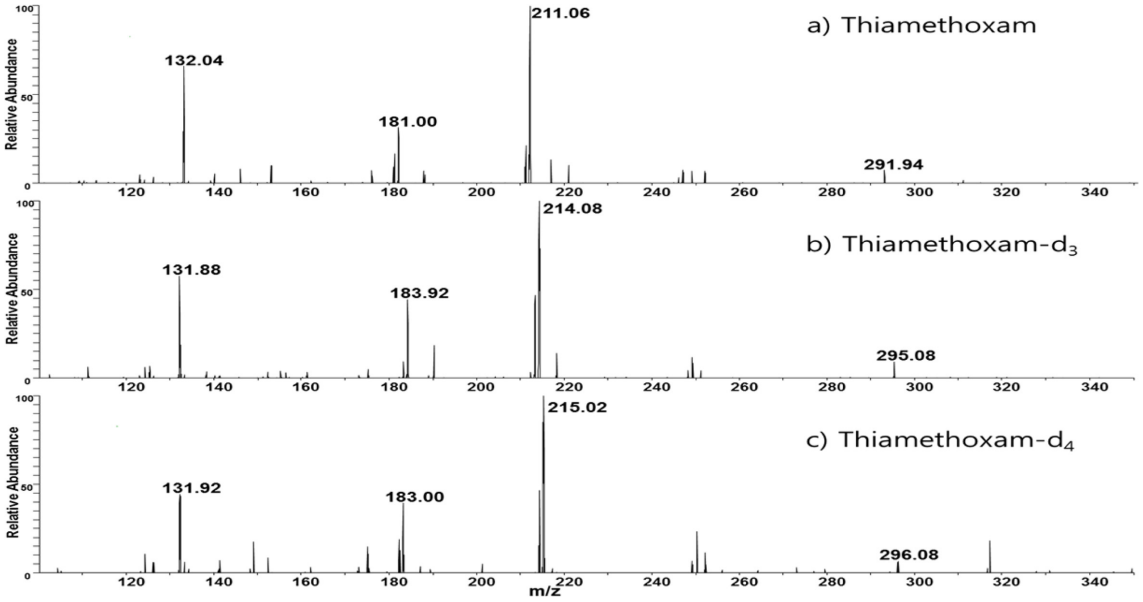


Figure 1. a) MS/MS spectrum of native thiamethoxam, b) MS/MS spectrum of thiamethoxam-d_3, c) MS/MS spectrum of thiamethoxam-d_4.

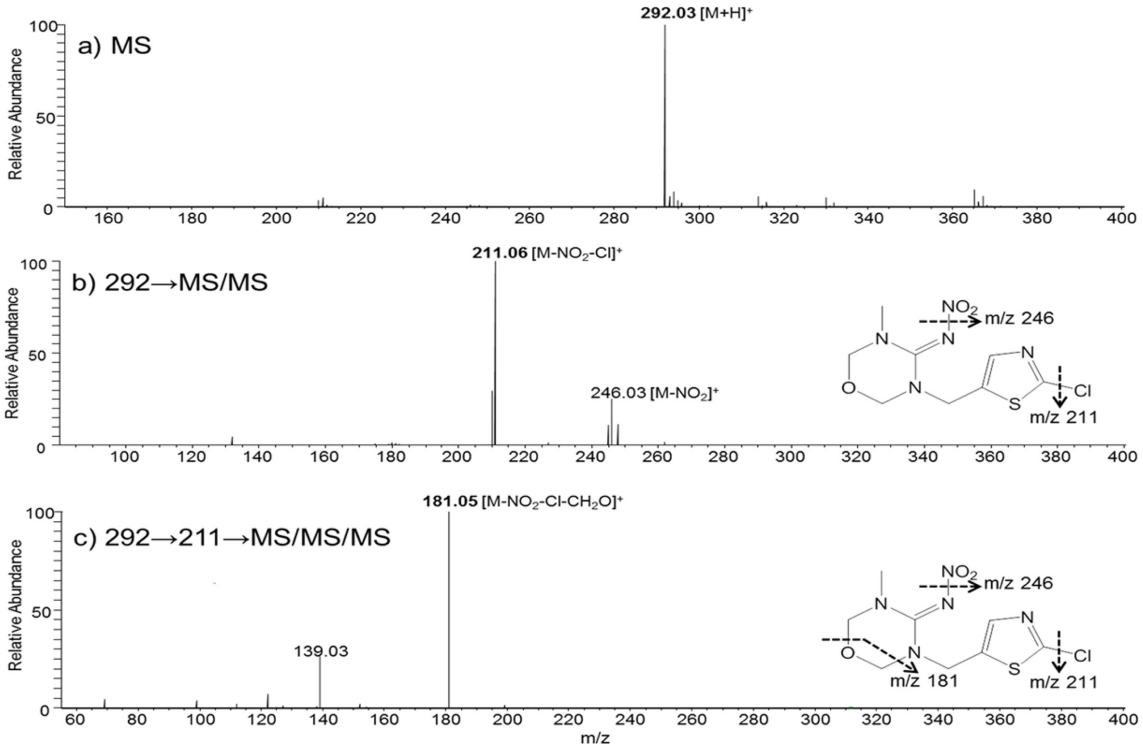


Figure 2. a) MS spectrum of, b) MS/MS spectrum from *m/z* 292 c) MS/MS/MS spectrum from *m/z* 292→211 of thiamethoxam by LC-LTQ-Orbitrap

z 296 and a fragment peak at *m/z* 183. The spectrum is shown in Figure 1 (c). Table 1 shows the *m/z* values of thiamethoxam, thiamethoxam-d_3, and thiamethoxam-d_4 agreed with those of the proposed candidate pathway. This indicated that the proposed pathway was valid as were the thiamethoxam peak assignment in the MS/MS data.

MS/MS/MS analysis

However, we still doubted the proposed candidate fragmentation pathway because there are no references. We considered other evidence for the assurance of the candidate pathway. We asked MS/MS/MS spectrum of thiamethoxam to Korea Basic Science Institute. Figure 2 (a) is a mass spectrum of thiamethoxam and shows a parent peak at *m/z* 292. Figure 2 (b) is an MS/MS spectrum of the peak at *m/z* 292 producing fragment peaks at *m/z* 211 and *m/z* 246. The peak at *m/z* 246 is the loss of NO_2 from the parent ion and the peak at *m/z* 211 is the loss of NO_2 and Cl. Figure 2 (c) is a MS/MS/MS spectrum of *m/z* 292 → 211 channel producing two fragment peaks at *m/z* 181 and *m/z* 139. The peak at *m/z* 181 was the fragment peak from *m/z* 211 due to the loss of CH_2O. According to the published pathways, the peaks at *m/z* 181 and *m/z* 211 should fragment simultaneously. However, the MS/MS/MS results indicated that the peak of *m/z* 181 was a consequent fragmentation of the peak at *m/z* 211. The peak at *m/z* 139 was not seen any other spectra from our data and or the literature. This requires further study. Finally, the MS/MS/MS of thiamethoxam suggested that the proposed fragmentation pathway was valid and could be used to assign the fragment peaks of thiamethosam.

Conclusions

To develope the IDMS method, conditions of MS/MS for thiamethoxam and its isotope analogue, thiamethoxam-d_3 were optimized. During optimization, it was observed that fragment peaks of MS/MS spectrum of isotope analogue, thiamethoxam-d_3 did not agree with the spectrum of thiamethoxam as expected based on the literature. We proposed a candidate pathway for these fragment peaks. To validate this novel pathway, another thiamethoxam isotope analogue, thiamethoxam-d_4 was obtained and inspected. Peaks in both MS/MS spectrum of thiamethoxam-d_3 and thiamethoxam-d_4 exactly agreed with the calculated peaks by the proposed pathway. It indicated that the proposed fragmentation pathway was valid to assign the fragment peaks. The MS/MS/MS spectrum of thiamathoxam showed that the origin of the peak at m/z181 was the peak at *m/z* 211, which means that the fragmentation pathyway by previous literature did not agree with the fragment peaks. The results of MS/MS/MS analysis of thiamethoxam supports the proposed candidate fragmentation pathway.

Acknowledgments

This work was supported by the Korea Research Institute of Standards and Science under projects “Establishment of standard system in organic analysis.’

We thank Dr. Jin Young Kim and Dr. Ju Yeon Lee (Korea Basic Science Institute, Ochang Headquarter, Division of Bioconvergence Analysis) for the nano LC-LTQ-Orbitrap analysis.

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