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# Development of an isotope dilution liquid chromatography/tandem mass spectrometry method for the accurate determination of neonicotinoid pesticides, imidacloprid, clothianidin, and thiamethoxam in kimchi cabbage reference materials

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## Abstract

A method based on isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS) was established as a candidate reference method for accurate determination of neonicotinoid pesticides, imidacloprid, clothianidin, and thiamethoxam in kimchi cabbage. Their deuterated isotopes, imidacloprid- $d_4$ , clothianidin- $d_3$ , and thiamethoxam- $d_4$  were used as internal standards. The combination of HLB and Carb solid-phase extraction (SPE) cartridges was used to clean-up kimchi cabbage extracts. The ID-LC/MS/MS conditions were optimized with fortified kimchi cabbage samples for validation. Imidacloprid in the ERM-BC403 cucumber sample ( $0.627 \pm 0.026$ ) mg/kg was analyzed with the developed method, and the measured value ( $0.604 \pm 0.028$ ) mg/kg agreed within their uncertainties. The developed method was employed for the certification of kimchi cabbage reference materials prepared in this laboratory. The measured values of imidacloprid, clothianidin, and thiamethoxam are ( $0.860 \pm 0.020$ ) mg/kg, ( $0.524 \pm 0.012$ ) mg/kg, ( $0.787 \pm 0.014$ ) mg/kg, respectively. The standard deviation of the measured values for ten bottles was < 1%, and the measured values after one year agreed with their first measurements indicating reliable repeatability and reproducibility of the developed method.

**Keywords:** Neonicotinoid pesticides, Imidacloprid, Clothianidin, Thiamethoxam, Matrix effects, Isotope dilution mass spectrometry (ID-MS), Reference materials

## Introduction

Neonicotinoid pesticides are a group of relatively new synthetic insecticides released in the 1990s and have become the most widely used class of insecticides worldwide (Berheim et al. 2019; Craddock et al. 2019; Elbert et al. 2008). Their chemical structures are similar to

those of nicotine, and they disrupt the nervous system of insects by acting on nicotinic acetylcholine receptors leading to paralysis and death (Bass et al. 2015; Maiefisch et al. 2001; Uneme 2011). In particular, neonicotinoid insecticides can be treated as a seed dressing; they migrate in the sap to all parts of the plant and provide protection against insects (Elbert et al. 2008; Whitehorn et al. 2012). Thus, they are very effective as insecticides throughout the life cycle of plants. Another major advantage of neonicotinoid insecticides is relatively low toxicity to mammals and humans (Goulson 2013; Nauen and

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Denholm 2005; Rundlöf et al. 2015; Tomizawa and Casida 2005). Despite these advantages, neonicotinoid pesticides are in the middle of a hot issue. It has been reported that neonicotinoid pesticides are a suspect causing a 'colony collapse disorder (CCD)' of honey bees in Europe, the USA, and Japan (Fairbrother et al. 2014; Henry et al. 2012; Ratnieks and Carreck 2010; Taniguchi et al. 2012; Whitehorn et al. 2012; Woodcock et al. 2016; Zeng et al. 2013). In spring 2013, the European Commission enacted a two-year moratorium on imidacloprid, thiamethoxam, and clothianidin treatment for bee-attractive crops. Finally, in April 2018, the European Parliament voted for a complete and permanent ban on all outdoor uses of the three most commonly used neonicotinoid pesticides (Commission 2018; European Food Safety Authority 2018). Most recently, the US Environmental Protection Agency restricted 12 products containing neonicotinoids in 2019 (USEPA 2020a, 2020b). Therefore, it is very important to accurately and precisely determine the levels of these three neonicotinoids in crops.

Because of the low volatility, thermolability, and high polarity of these compounds, direct analysis by gas chromatography (GC) has proved unsuitable, but a few analyses were performed after hydrolysis or derivatization (MacDonald and Meyer 1998; Navalón et al. 1997). In some instances, analyses of neonicotinoid pesticides were performed by high-pressure liquid chromatography (HPLC) coupled with diode array detection (Campillo et al. 2013; Jovanov et al. 2015). However, nowadays the most common method is HPLC coupled with tandem mass spectrometry (MS/MS) due to its high selectivity and sensitivity (Dankyi et al. 2015; Iwafune et al. 2014; Liu et al. 2010; Ma et al. 2020; Xiao et al. 2013).

Despite the advanced nature of the LC/MS/MS tool, the most pernicious fundamental problem with powerful approaches is their susceptibility to matrix effects, which adversely affect quantification when analyzing complex samples (Gosetti et al. 2010; Niessen et al. 2006; Trufelli et al. 2011). Matrix effects are caused by co-eluted materials, which are introduced during the sample preparation procedure and can be very complicated and unexpected. Matrix effects are responsible for poor and unreliable data in a quantitative assay; they can heavily affect the reproducibility, linearity, and accuracy of the method and lead to erroneous quantitation. To overcome the matrix effects, clean-up steps in the sample preparation procedure are necessary. However, the clean-up procedure leads to another obstacle, because it results in poor recovery and precision of pesticide analysis.

The Korea Research Institute of Standards and Science (KRISS), as the National Metrology Institute (NMI) of Korea, has endeavored to reduce biases to facilitate more accurate and precise quantitation. Matrix effects are one

of the main bias factors in food analysis. To reduce the matrix effects, we have used the clean-up procedures extensively as well as used isotope analogues of each analyte as internal standards based on the isotope-dilution mass spectrometry (ID-MS) method (Ahn et al. 2020). However, the ID-MS method does not completely compensate for matrix effects. If the isotope analogues have different retention time from the native one, they experience different matrix circumstances. (Lee et al. 2017; Lindegardh et al. 2008).

In this study, the ID-LC/MS/MS method for the accurate determination of three neonicotinoid pesticides, imidacloprid, clothianidin, and thiamethoxam, in kimchi cabbage was established as a candidate reference method. The clean-up procedure, LC, and MS conditions were optimized for the ID-LC/MS/MS method and its validation. To validate this method, fortified kimchi cabbage samples with three pesticides were analyzed by the optimized ID-LC/MS/MS method. Another validation was accomplished with the ERM-BC403 (Joint Research Center, EC) cucumber sample containing imidacloprid. The cucumber sample is only for imidacloprid; thus, kimchi cabbage reference materials including imidacloprid, clothianidin, and thiamethoxam were prepared in this laboratory and characterized via the established LC/MS/MS method.

## Methods and materials

### Chemicals

Imidacloprid, clothianidin, and thiamethoxam were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Purity assays of these pesticides were performed according to protocols developed in this laboratory at the National Metrology Institute of Korea. Details of the purity assay have been described in previous studies (Kim et al. 2013; Lee and Kim 2014). Briefly, LC/UV analysis for structure-related impurities, Karl-Fisher titration for water content, thermogravimetric analysis for non-volatile impurities, and head-space GC/MS for residual solvents were performed, respectively, and the chemicals were subsequently used without further purification. The purity of imidacloprid, clothianidin, and thiamethoxam was determined as  $(99.5 \pm 0.3)\%$ ,  $(99.4 \pm 0.3)\%$ , and  $(99.6 \pm 0.1)\%$ , respectively. Isotope analogues, imidacloprid- $d_4$ , clothianidin- $d_3$ , and thiamethoxam- $d_4$ , were obtained from LGC (Teddington, UK). Ammonium acetate for the aqueous mobile phase was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride and sodium sulfate for sample preparation procedure were also purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade organic solvents, methanol, and acetonitrile were obtained from Burdick and Jackson

(Musekegon, MI, USA). Water was purified with a Millipore Milli-Q system at  $18.2 \text{ M}\Omega/\text{cm}^2$ .

#### Calibration standard solutions

All solutions were prepared with 5 mM ammonium acetate aqueous solution and stored at  $-20^\circ\text{C}$ . A standard solution mixture containing the three pesticides was prepared gravimetrically at a mass fraction of 10 mg/kg. An isotope standard solution mixture containing imidacloprid- $d_4$ , clothianidin- $d_3$ , and thiamethoxam- $d_4$  was gravimetrically prepared at a mass fraction of 10 mg/kg. An isotope ratio standard solution was prepared gravimetrically by mixing the standard solution and isotope standard solutions at an isotope ratio of 1:1. Subsequently, the isotope ratio standard solutions were appropriately diluted for the analysis.

#### Sample preparation and clean-up

Kimchi cabbage is an ingredient of kimchi. Kimchi cabbages were obtained from a Korean farm and prepared in powder form for use as reference materials. Thus, the sample preparation procedure was optimized for kimchi cabbage powder. One gram of kimchi cabbage powder was weighed into a 50-mL conical tube. The sample was spiked with the isotope standard solution, followed by 10 mL of water for reconstitution to the corresponding raw materials. The extraction of target pesticides was achieved by adding 20 mL acetonitrile to the sample followed by mixing on a mechanical shaker for 10 min. The extract was separated with water and acetonitrile layers using 3 g NaCl, and only the acetonitrile (upper) layer was transferred to a new tube. The clean-up protocols varied based on the type of solid-phase extraction (SPE) cartridges employed. The extract (acetonitrile layer) was dried under  $\text{N}_2$  gas and then reconstituted with water for the HLB cartridge, which was conditioned with 5 mL methanol and 5 mL water. After loading the sample extract, the cartridge was washed with 5 mL water and eluted with 5 mL methanol. For the Florisil cartridge, the extract (acetonitrile layer) was dried under  $\text{N}_2$  gas and then reconstituted with 5 mL dichloromethane. The Florisil cartridge was conditioned with 5 mL dichloromethane before sample loading. The sample extract was loaded and eluted with 5 mL of acetone: dichloromethane mixture (20:80, v/v). Eluents from both the loading and eluting stages were collected together. The eluent from the HLB or Florisil cartridge was followed by a Carb (carbon) cartridge to reduce the pigments from the kimchi cabbage sample. The Carb cartridge was conditioned with 5 mL methanol. The eluent from the HLB or Florisil cartridge was loaded and eluted with 5 mL methanol. The eluents from both loading and eluting were collected together. For the Carb/ $\text{NH}_2$  cartridge, 5 mL toluene was

added to the extract (acetonitrile layer) to yield a 3:1 ratio (acetonitrile: toluene, v/v). After conditioning the Carb/ $\text{NH}_2$  cartridge with 5 mL of an acetonitrile: toluene mixture (3:1, v/v), the sample extract solution (in acetonitrile: toluene = 3:1) was loaded and eluted with 10 mL of an acetonitrile: toluene mixture (3:1, v/v). After clean-up, all eluents were evaporated under  $\text{N}_2$  gas and then reconstituted with 1 mL of a 5 mM ammonium acetate aqueous solution (aqueous mobile phase) for analysis by LC/MS/MS.

#### LC/MS/MS analysis

The LC/MS/MS system consisted of a Triple Quad 6500 triple quadrupole mass spectrometer (AB Sciex, Foster City, USA) interfaced with a Waters Acquity UPLC system (Manchester, UK) through an electrospray ionization (ESI). Separation was accomplished with an Acquity UPLC BEH C18 column ( $1.7 \mu\text{m}$ ,  $2.1 \times 100 \text{ mm}$ ) connected to an Acquity UPLC BEH C18 VanGuard ( $1.7 \mu\text{m}$ ,  $2.1 \times 5 \text{ mm}$ ) as a guard column. The injection volume was 10  $\mu\text{L}$ . The mobile phases started with 70% A of 5 mM ammonium acetate in water and 30% B of methanol for 1 min. The initial mobile phases changed to 45% B in 5 min, and then, the column was washed with 100% B for 5 min, followed by a return to the initial phase for 5 min. The flow rate was set to 0.2 mL/min. Analyst software was used for instrument control and data processing. The mass spectrum was obtained in positive ion mode and selected reaction monitoring (SRM) mode. The ion spray voltage was 5000 V.

#### Calculation and uncertainty

The mass fractions of imidacloprid, clothianidin, and thiamethoxam ( $C_{\text{sample}}$ ) in food samples were determined using the ID-LC/MS/MS analysis, as follows:

$$C_{\text{sample}} = \frac{M_{\text{is-sol,spiked}} \cdot \text{AR}_{\text{sample}} \cdot M_{\text{s-sol,std.mix}} \cdot C_{\text{s-sol}}}{M_{\text{sample}} \cdot \text{AR}_{\text{std.mix}} \cdot M_{\text{is-sol,std.mix}}}$$

where  $C_{\text{sample}}$  is the mass fraction of the analyte (target pesticides);  $M_{\text{is-sol,spiked}}$  corresponds to the mass the isotope-labeled analyte added to the sample;  $\text{AR}_{\text{sample}}$  corresponds to the observed area ratio of the analyte and the isotope-labeled analyte from the LC/MS/MS analysis of the sample;  $M_{\text{s-sol,std.mix}}$  and  $M_{\text{is-sol,std.mix}}$  correspond to the masses of the pesticide standard solution and the isotope-labeled standard solution, respectively, which were added to the isotope ratio standard solution;  $C_{\text{s-sol}}$  is the concentration of the analyte in the standard solution;  $M_{\text{sample}}$  is the mass of the sample, and  $\text{AR}_{\text{std.mix}}$  corresponds to the observed area ratio of the analyte and the isotope-labeled analyte from LC/MS/MS analysis of the isotope ratio standard solution. The uncertainty of the measured values was evaluated

according to the protocol described in previous studies (Choi et al. 2003a, 2003b; Kim et al. 2010) which follows the Guide to the Expression of Uncertainty in Measurements (JCGM, 2008). Expanded uncertainties from Table 1 were obtained by combining the relative standard deviations of multiple measurements for the isotope ratio standard solution and for samples. The expanded uncertainties of the measured value for imidacloprid in ERM-BC403 and kimchi cabbage reference materials were calculated by combining systematic and random effects. The systematic uncertainty was composed of uncertainties for the purity assay of three pesticides (imidacloprid, clothianidin, and thiamethoxam), uncertainties for the preparation of standard solutions, and uncertainties for relative standard deviations of multiple measurements for isotope ratio standard solutions. The random effect was the relative standard deviation of the mass fractions of four sub-samplings from the ERM-BC403 sample and ten sub-samplings from kimchi cabbage reference materials.

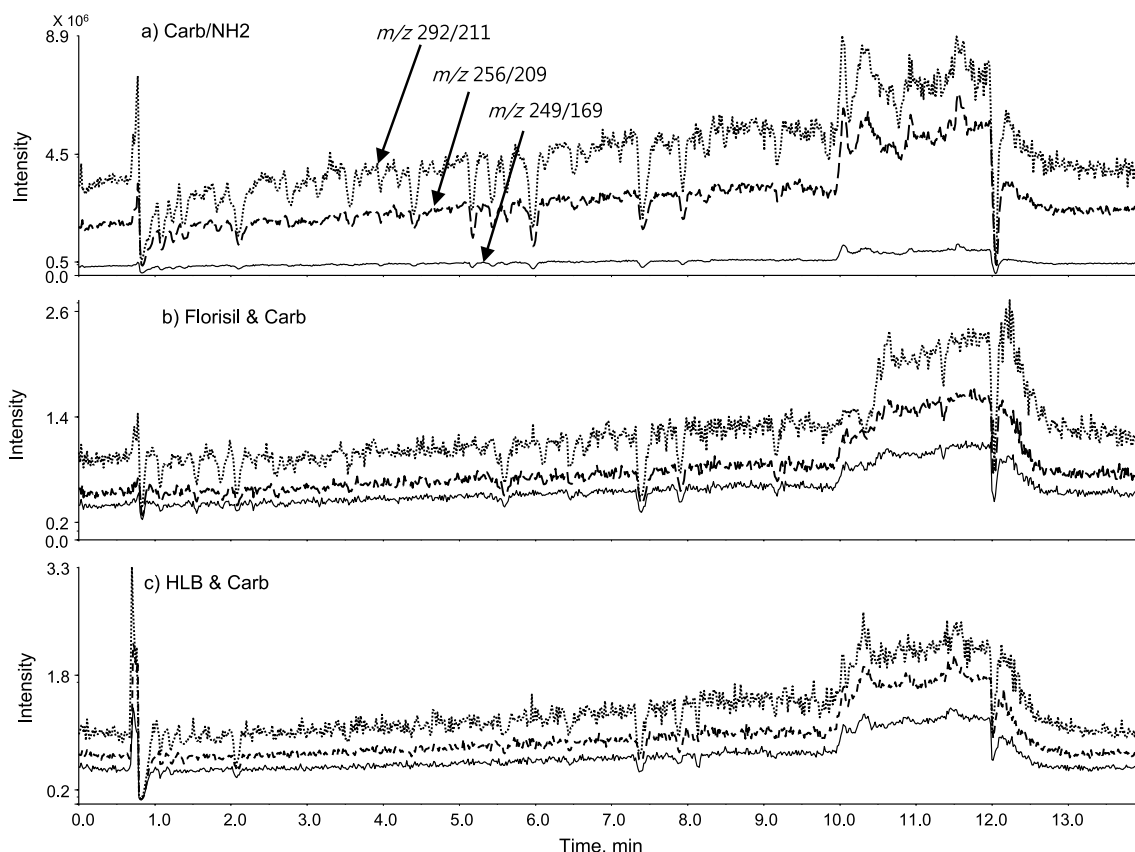
### Preparation of kimchi cabbage reference materials

Kimchi cabbage powder was prepared by protocols developed in this laboratory described elsewhere (Ahn et al. 2020). Briefly, kimchi cabbage was purchased from a local cabbage farm and they were freeze-dried and pulverized. After pulverizing, the 50–250- $\mu\text{m}$ -sized powder was collected by sieving. An aqueous solution including imidacloprid, clothianidin, and thiamethoxam was added into the kimchi cabbage powder. The powder then formed a paste which was freeze-dried, pulverized, and sieved again. 15 g of kimchi cabbage powder was bottled and then stored at  $-70^\circ\text{C}$  before analysis.

## Results and discussions

### MS/MS conditions

Mass spectra of the three pesticides and their isotope analogues were observed via direct infusion in positive ion mode and full scan mode. The protonated molecules  $[\text{M} + \text{H}]^+$  at  $m/z$  256 (260), 250 (253), and 292 (296) were dominant for imidacloprid (imidacloprid- $d_4$ ),

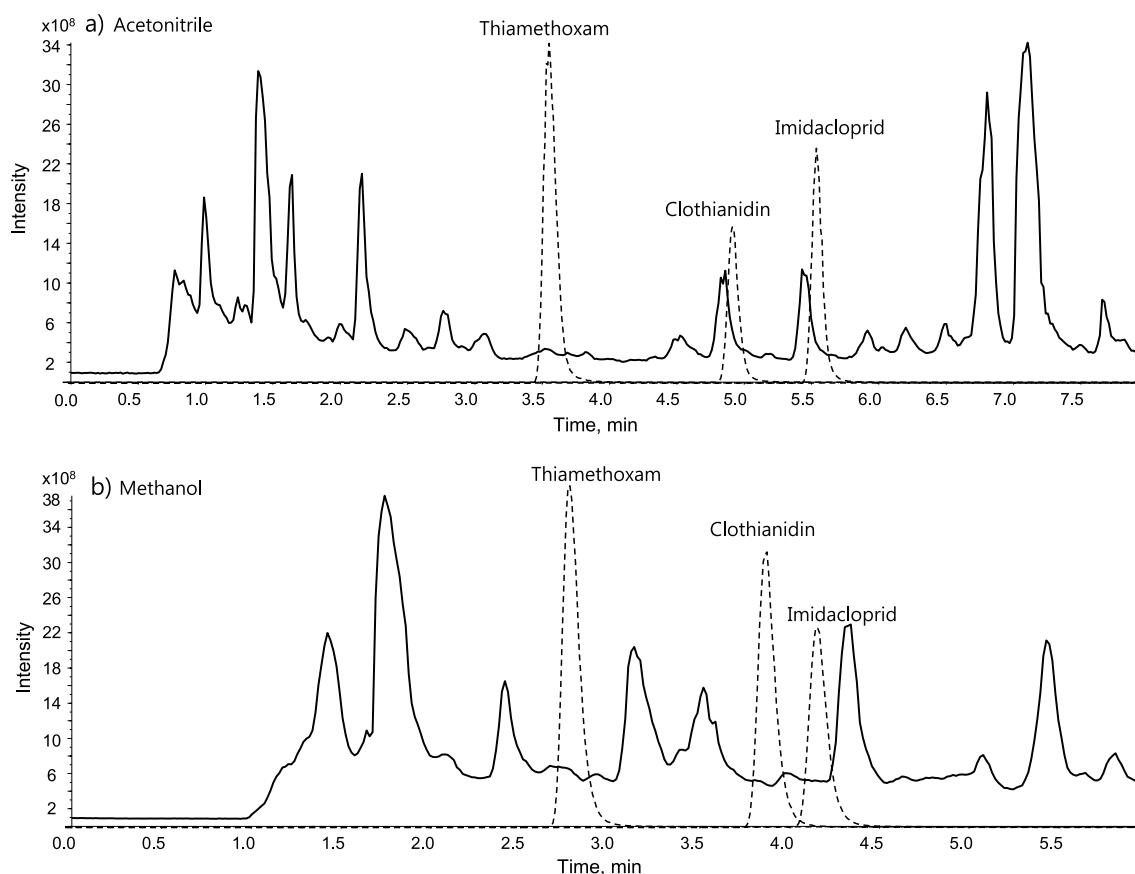


**Fig. 1** Post-column infusion profiles of imidacloprid (--- dashed line for  $m/z$  256  $\rightarrow$  209), clothianidin (— solid line for  $m/z$  249  $\rightarrow$  169), and thiamethoxam (..... dotted line for  $m/z$  292  $\rightarrow$  211) for the evaluation of matrix effects of kimchi cabbage powder after **a** Carb/ $\text{NH}_2$  SPE cartridge, **b** Florisil and Carb SPE cartridges, and **c** HLB and Carb SPE cartridges

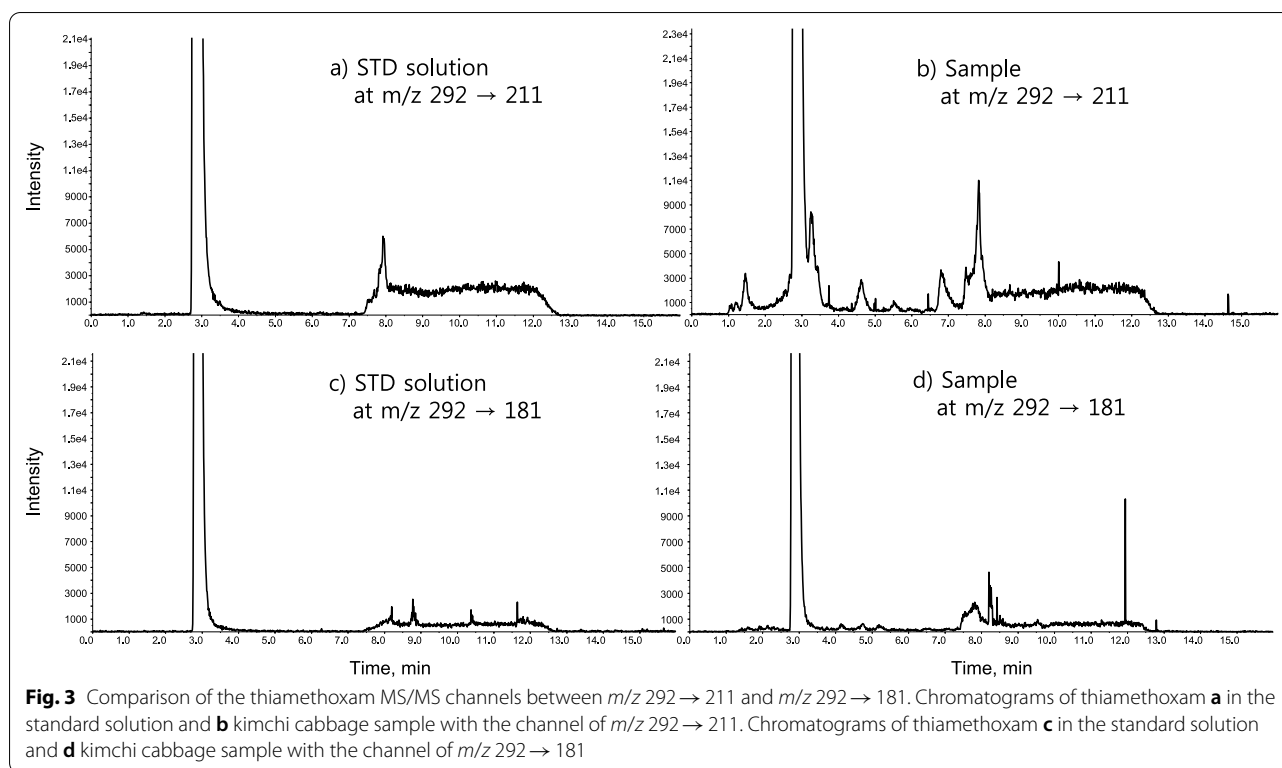
clothianidin (clothianidin- $d_3$ ), and thiamethoxam (thiamethoxam- $d_4$ ), respectively. To operate the multiple reaction monitoring (MRM) mode, collision-induced dissociation (CID) mass spectra were observed, and their conditions were optimized. The CID mass spectra of imidacloprid and imidacloprid- $d_4$  were dominated by fragment ions at  $m/z$  256  $\rightarrow$  209 and  $m/z$  260  $\rightarrow$  213, respectively, which were attributed to  $[M+H-HNO_2]^+$  with a collision energy (CE) of 21 V. The spectra of clothianidin and clothianidin- $d_3$  were dominated by fragment ions at  $m/z$  250  $\rightarrow$  169 and  $m/z$  253  $\rightarrow$  172, respectively, which were attributed to  $[M+H-NO_2-Cl]^+$  with a CE of 17 V. In the case of thiamethoxam and thiamethoxam- $d_4$ , dominant fragment ions were  $[M+H-NO_2-Cl]^+$  at  $m/z$  292  $\rightarrow$  211 and  $m/z$  296  $\rightarrow$  215 with a CE of 15 V. However, these peaks were affected by co-eluted materials from food samples; thus, the final selected fragment ions were  $[M+H-Cl-NO_2-CH_2O]^+$  at  $m/z$  292  $\rightarrow$  181 and 296  $\rightarrow$  183 with a CE of 31 V. The reason for the change in the CID channel for thiamethoxam is described later in this section.

### Selection of solid-phase extraction

Recently, QuEChERS method for sample cleaning has been commonly used in the test laboratories because it is quick, easy, cheap, effective, rugged, and safe. Instead of these merits, this method was not efficient to remove co-eluting materials from sample matrices for accurate quantitation of some pesticides (Takamits et al. 2016). Thus, an SPE column was still used for cleaning up co-eluted materials from food samples. However, it is not easy to select an appropriate SPE column considering both analytes and co-eluted interferences. Three SPE columns (Carb/ $NH_2$ , HLB and Carb, Florisil and Carb) were selected for background profiles (post-column infusion profile) because they are commonly used for pesticide analyses. The Carb/ $NH_2$  column is a two-in-one column; thus, it is effective for managing and reducing time. Florisil or HLB was not enough to remove pigments from kimchi cabbage, but Florisil or HLB cartridges are not commercially available as two-in-one columns with Carb cartridge. Thus, the Florisil or HLB cartridges were followed by a Carb cartridge. The Carb cartridge is known



**Fig. 2** Chromatograms of scan mode (solid line) for the eluent from kimchi cabbage (blank kimchi cabbage) and SRM mode (dashed line) of standard solution mixture of imidacloprid, clothianidin, and thiamethoxam with **a** acetonitrile and **b** methanol as an organic mobile phase of LC



**Fig. 3** Comparison of the thiamethoxam MS/MS channels between  $m/z$  292  $\rightarrow$  211 and  $m/z$  292  $\rightarrow$  181. Chromatograms of thiamethoxam **a** in the standard solution and **b** kimchi cabbage sample with the channel of  $m/z$  292  $\rightarrow$  211. Chromatograms of thiamethoxam **c** in the standard solution and **d** kimchi cabbage sample with the channel of  $m/z$  292  $\rightarrow$  181

as an effective SPE cartridge for reducing pigments from food samples. Figure 1 shows the post-column infusion profiles which explain how the analyte ions are affected by co-eluted materials from sample matrix. The protocols for post-column infusion profiles are described in previous studies (Ahn et al. 2017; Lee et al. 2017; Lindegardh et al. 2008). Briefly, samples were prepared by extraction and clean-up processes using various SPE columns. These samples were injected onto the analyte column and run to the ESI source of MS. Before the ESI source, the LC eluent was infused with a constant flow of standard solution of analytes via a T-connector. The chromatograms had a higher background by infused standard solution of analytes. These background profiles were affected by the co-eluted materials from kimchi cabbage samples during the sample preparation procedure. Three chromatograms in Fig. 1a–c show the profiles of channels at  $m/z$  292/211 for thiamethoxam (dotted line),  $m/z$  256/209 for imidacloprid (dashed line), and  $m/z$  249/169 for clothianidin (solid line), respectively, after (a) Carb/ $\text{NH}_2$ , (b) Florisil & Carb, and (c) HLB & Carb SPE cartridges, respectively. If the analyte ions are not affected by co-eluted materials, the profile lines would be straight, but if affected, they would be up (enhanced) and down (suppressed). The post-column profiles were suppressed or enhanced during solvent gradient time around 1–10 min of chromatogram. Both chromatograms (c) for HLB &

Carb cartridges were clearest than those of the Carb/ $\text{NH}_2$  cartridge and Florisil & Carb cartridges. The final selection was the combination of HLB & Carb cartridges as it allowed for more efficient use of time.

#### Optimization LC/MS conditions

To reduce the effects of co-eluted materials from kimchi cabbage, various gradient conditions were applied to the LC separation. However, no significant differences in the results were observed (data not shown). The organic mobile phase of the LC was then changed from acetonitrile to methanol. The solid line in Fig. 2 shows the scan mode chromatograms for the eluent from kimchi cabbage without the three pesticides. Peaks (dotted line) in chromatograms correspond to three analytes in standard solutions obtained separately under the respective LC conditions. The chromatogram (a) in Fig. 2 was obtained with acetonitrile, whereas (b) was obtained with methanol. Although the peaks of clothianidin and imidacloprid are partly overlaid by the co-eluent peaks from the matrix in Fig. 2a, all three analyte peaks are separated from the co-eluent peaks in Fig. 2b. The intensity of clothianidin and imidacloprid changed slightly; however, in the ID-LC/MS/MS method, the change in intensity did not affect quantitation. Thus, the analysis could be performed with methanol as an organic solvent.



**Table 1** Measurement results of gravimetrically fortified samples to kimchi cabbage powder by ID-LC/MS/MS at various mass fractions

Analytes	Fortified value (mg/kg)	Measured value (mg/kg) <sup>a</sup>	Difference (%) <sup>b</sup>
<i>Imidacloprid</i>			
#1	2.482	2.503 ± 0.033	0.85
#2	2.481	2.503 ± 0.025	0.85
#3	0.625	0.620 ± 0.006	−0.88
#4	0.491	0.487 ± 0.005	−0.89
#5	0.245	0.246 ± 0.003	0.78
#6	0.246	0.246 ± 0.003	0.21
<i>Clothianidin</i>			
#1	2.923	2.998 ± 0.032	2.58
#2	2.921	3.002 ± 0.036	2.77
#3	0.736	0.735 ± 0.004	−0.09
#4	0.578	0.583 ± 0.003	0.72
#5	0.288	0.290 ± 0.002	0.55
#6	0.289	0.290 ± 0.004	0.31
<i>Thiamethoxam</i>			
#1	2.760	2.757 ± 0.034	−0.10
#2	2.758	2.765 ± 0.036	0.22
#3	0.695	0.701 ± 0.008	0.91
#4	0.546	0.550 ± 0.006	0.75
#5	0.272	0.276 ± 0.004	1.67
#6	0.273	0.277 ± 0.003	1.61

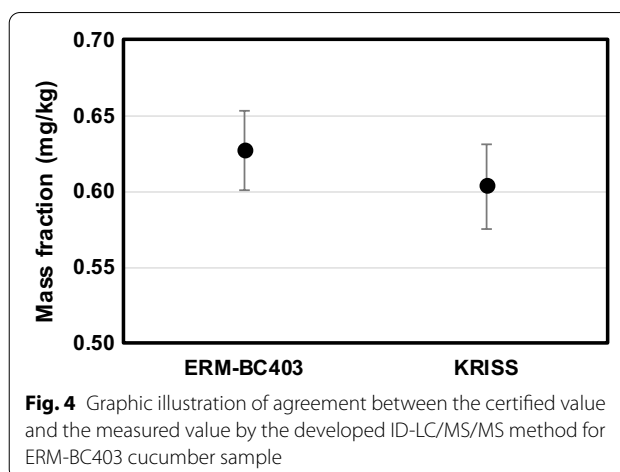
<sup>a</sup> The number after '±' is the expanded uncertainty of the preceding value with the level of confidence of 95%

<sup>b</sup> Relative difference of the measured value from the gravimetrically fortified value

We examined the peak of thiamethoxam from fortified kimchi cabbage. As shown in Fig. 3, the peak of thiamethoxam in the standard solution (a) appeared clean and sharp, whereas the peak of thiamethoxam in the eluent from kimchi cabbage (b) showed a fronting tail and a shoulder caused by co-eluting materials with  $m/z$  292 → 211 channel. These features provided a bias in the peak area of thiamethoxam. To avoid the peaks of co-eluted materials around the thiamethoxam peak, a new channel of thiamethoxam was adopted from  $m/z$  211 to 181 for fragmentation with a CE of 31 V. The change in the channel results in chromatogram (d) in Fig. 3 with a sharp and clear peak of thiamethoxam. Therefore, the channel from  $m/z$  211–181 was selected for the analysis of thiamethoxam.

#### Validation of optimized conditions

Analysis was carried out with the optimized conditions described in the *MS/MS conditions* section above. The results for the fortified samples in kimchi cabbage containing various mass fractions of the three target

**Fig. 4** Graphic illustration of agreement between the certified value and the measured value by the developed ID-LC/MS/MS method for ERM-BC403 cucumber sample

pesticides are listed in Table 1. Differences indicate the ratio percentage of fortified mass fractions to kimchi cabbage samples and measured mass fractions by LC/MS/MS. In the range of 0.2–3.0 mg/kg fortified mass fraction, the difference was around 1–3%. Another way to validate the developed method is analysis of certified reference materials (CRMs). There is no commercial CRM containing the three target pesticides, but a CRM containing imidacloprid in an ERM-BC403 cucumber sample is available in commercial. Thus, this CRM was analyzed to determine the mass fraction for imidacloprid by the developed ID-LC/MS/MS method. The certified value of imidacloprid in ERM-BC403 was (0.627 ± 0.026) mg/kg. This value agreed with the measured value, (0.604 ± 0.028) mg/kg, indicating the validity of the analytical method (Fig. 4).

The limits of detection (LODs) of the instrumental analysis and the limits of quantitation (LOQs) were tested at series of levels in the range of 0.5–5 µg/kg of calibration standard solutions with each analyte at series of level in 0.5–5 µg/kg analyzed by LC/MS/MS. The LODs (signal-to-noise ratio of 3) for imidacloprid, clothianidin, and thiamethoxam were 0.2, 0.1, and 0.1 µg/kg, respectively. The LOQs (signal-to-noise ratio of 10) for three pesticides were estimated to be 1.5 µg/kg in kimchi cabbage, respectively.

#### Repeatability and reproducibility of the developed method: preparation of reference materials

Because the ERM-BC403 contains only imidacloprid, kimchi cabbage reference materials were prepared as described in “*Materials and methods*” section to ensure the validation of the developed method. Ten bottles of kimchi cabbage reference materials were selected with a regular order of filling, including the first and the last ones. One sub-sampling from each of the ten bottles

**Table 2** Measurement values of kimchi cabbage reference materials by the developed ID-LC/MS/MS method

	Imidacloprid	Chlothianidin	Thiamethoxam
<i>Period 1</i>			
Average values (mg/kg, $n = 10$ )	0.860	0.524	0.787
Standard deviation (%)	0.51	0.40	0.46
Expanded uncertainty (mg/kg)	0.020	0.012	0.014
<i>Period 2</i>			
Average values (mg/kg, $n = 4$ )	0.837	0.514	0.774
Standard deviation (%)	0.31	0.27	0.37
Expanded uncertainty (mg/kg)	0.034	0.015	0.023

was sued for the certification analysis. These ten sub-samplings were analyzed by the developed ID-LC/MS/MS method. The measurement results of ten samplings and their uncertainty sources are listed in the Additional file 1: Tables S1–S3. Table 2 shows the average values of ten bottles and expanded uncertainties with imidacloprid ( $0.860 \pm 0.020$ ) mg/kg, clothianidin ( $0.524 \pm 0.012$ ) mg/kg, and thiamethoxam ( $0.787 \pm 0.014$ ) mg/kg. The standard deviations of ten bottles of imidacloprid, clothianidin, and thiamethoxam were 0.51%, 0.40%, and 0.46%, respectively. These values were all below 1%, indicating that the repeatability of the developed ID-LC/MS/MS method was acceptable, and a graphical description is shown in Additional file 1: Fig. S1. One year after the first measurement (period 1), the same analysis was performed using four bottles (period 2). The measurement values of period 2 agreed with those of period 1 within their overall uncertainties and the difference between the values of two periods were below 2%, indicating that the reproducibility of this method was also acceptable and a graphical description is shown in Additional file 1: Fig. S2. Analysis with multiple sub-samplings and measurements within two different time periods showed reliable repeatability and reproducibility of the developed method.

## Conclusions

An ID-LC/MS/MS method was established for the accurate determination of neonicotinoid pesticides, imidacloprid, clothianidin, and thiamethoxam in kimchi cabbage. To reduce the interference of co-eluting materials from kimchi cabbage samples in the determination of pesticide levels, a combination of HLB and Carb cartridges was selected for the clean-up step in sample preparation. Methanol as an organic mobile phase of LC was used to separate co-eluting materials that overlapped with the analyte pesticide peaks. In the case of thiamethoxam, an  $m/z$  292  $\rightarrow$  181 MS/MS channel was chosen rather than an  $m/z$  292  $\rightarrow$  211 channel for a clean baseline around the main peak. The developed method was validated with fortified kimchi cabbage samples. Various fortified values

ranging from 0.2 to 3.0 mg/kg were tested, and the results showed 1–3% differences among the measured and fortified values. The BCR-403 cucumber sample containing only imidacloprid was analyzed using the developed method. The measured value was ( $0.604 \pm 0.028$ ) mg/kg and the certified value was ( $0.627 \pm 0.026$ ) mg/kg, indicating that they agreed within their own uncertainties. Kimchi cabbage reference materials were prepared in this laboratory and determined by the developed ID-LC/MS/MS method. The measured values were ( $0.860 \pm 0.020$ ) mg/kg for imidacloprid, ( $0.524 \pm 0.012$ ) mg/kg for clothianidin, and ( $0.787 \pm 0.014$ ) mg/kg for thiamethoxam. The standard deviations of the values for the ten sub-samplings were 0.51%, 0.40%, and 0.46%, respectively. One year after the first analysis, three pesticides were measured again with four bottles of reference materials, and these values agreed with the first measurement within their uncertainties. These results indicate that the repeatability and the reproducibility of the developed method are suitable for the characterization of reference materials as a candidate reference method. Kimchi cabbage reference materials can be disseminated after further stability monitoring and the KRISS CRM process.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40543-022-00319-4>.

**Additional file 1. Fig. S1.** Graphic illustration of measurement results of 10 bottles of kimchi cabbage reference materials (RM). Error bars are the expanded measurement uncertainties ( $k \cdot u_j$ ) of the unit, where  $k$  is the coverage factor with the confidence level of 95 %, is ranging from 2.3 ~ 2.8 depending on data points. **Fig. S2.** Comparison of the measurement values at different time periods with the developed ID-LC/MS/MS method.

## Acknowledgements

This research was supported by the Korea Research Institute of Standards and Science under the project 'Establishment of standard system in organic analysis,' Grant 20011034.

## Authors' contributions

SA involved in conceptualization, methodology, investigation, writing—original draft and editing, SS took part in methodology, validation, investigation,



writing—figures and tables, BK involved in project administration, and KC took part in methodology—instrument, resources. All authors read and approved the final manuscript.

#### Funding

Not applicable.

#### Availability of data and materials

Not applicable.

#### Declarations

#### Competing interests

All authors declare that they have no competing of interest.

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Received: 6 December 2021 Accepted: 15 March 2022

Published online: 23 March 2022

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