



Accurate Determination of Carbaryl, Carbofuran and Carbendazim in Vegetables by Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry

Seonghee Ahn¹ · Ji-Young Lee² · Byungjoo Kim¹

Received: 12 June 2020 / Revised: 7 September 2020 / Accepted: 10 October 2020 / Published online: 31 October 2020
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Abstract

An analytical method for the accurate determination of carbamate pesticides, carbaryl, carbendazim, and carbofuran, in vegetables was established based on the isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS) technique as a candidate reference method. For sample clean-up, a Carb/LC-NH₂ solid-phase extraction (SPE) cartridge was employed to ensure an efficient recovery and color removal from the vegetable samples. Isotope analogues, ¹³C₆-carbaryl, carbendazim-*d*₄, and ¹³C₆-carbofuran, were used as internal standards, respectively. This method was validated by measuring samples fortified with the three target analytes into vegetable samples and prepared homogeneous Korean cabbage powder samples in this laboratory. All results of the measured values agreed with the fortified values within their uncertainties. Repeatability and reproducibility of the method were both approximately 2%. As commercially available CRM contains only carbendazim, its content was analyzed by the developed method. The measured value of carbendazim in CRM was (0.0724 ± 0.0013) mg/kg agreeing with the certified value (0.074 ± 0.004) mg/kg. These validation data indicate the developed ID-LC/MS/MS method is reproducible, repeatable and reliable to be as a candidate reference method for accurate determination of carbamate pesticides in vegetables.

Keywords Carbamate pesticide · Carbaryl · Carbofuran · Carbendazim · Isotope dilution mass spectrometry

Introduction

Carbamate pesticides are derived from carbamic acid [1, 2], which contains the –NH(CO)O– functional group. They act as acetylcholinesterase (AChE) inhibitors to prevent acetylcholine build up [3–5]. The mechanism of toxicity of carbamates is, therefore, similar to that of organophosphates, as they also inhibit AChE [6]; however, it has been reported that carbamate pesticides are relatively less toxic to humans than organophosphates and more rapidly degradable than organochlorines [7]. Such carbamate pesticides have a broad spectrum of activity in the areas of agriculture, horticulture

and even household use for home interiors or gardening [8]. In the case of carbendazim, it has been extensively used worldwide as a fungicide to protect vegetables and fruits from fungus and molds [9, 10]. Their great success of applications in various areas has led to a continued increase in the use of carbamate pesticides overall.

Although carbamate pesticides are known to be relatively less toxic, long-term exposure in humans, especially via inhalation and ingestion, has caused cholinesterase inhibition leading to the inactivation of this essential enzyme which is suspected of being a carcinogen and mutagen [11, 12]. Carbamate pesticides have also been reported to cause adverse effects on the male reproductive system and fertility issues in both humans and rats [13, 14]. Recently, in Korea, carbendazim was detected in a number of imported fruits including bananas from the Philippines and orange juice from Spain. In addition, due to their high polarity and water solubility, carbamate pesticides flow readily into the soil and rivers, thereby giving rise to environmental contamination [6, 15, 16]. As such, many nations and organizations (e.g., the EU,

✉ Seonghee Ahn
sahn@kriss.re.kr

¹ Division of Chemical and Biological Metrology, Korea Research Institute of Standards and Science, Yuseong, Daejeon 34113, Republic of Korea

² Present Address: Gyeongin Regional Office of Food and Drug Safety, Gwacheon, Gyeonggi-do 13809, Republic of Korea

Japan, and Korea) have established regulations with maximum residue limits (MRLs) of carbamate pesticides in various foods and food products, and so the development of a highly accurate and reliable method for measuring carbamate pesticides in food samples is necessary.

Traditionally, the analysis of carbamate pesticides has been accomplished by gas chromatography with derivatization [7, 17, 18] and HPLC with various detectors such as a fluorescence detector [19, 20], and an UV/Vis detector [17, 21–25]. To date, liquid chromatography coupled with mass spectrometry was employed to the analysis for carbamate pesticides with superior selectivity [26–29]. Recently pesticide residue analyses in food have more focused on a multi, quick and simultaneous method [30–33]. Of course, it is very important to analyze hundreds of pesticides from huge amount of food products in quick and simultaneous. However, these multiclass and multi-residue analyses in food compromise the accuracy of analysis [34]. This laboratory (Korea Research Institute of Standards and Science), which is the National Metrology Institute of Korea, has developed the method for accurate determination using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) as a higher order reference method which have metrological qualities with traceability to the International System (SI) units. The combination of isotope dilution mass spectrometry with chromatographic techniques is preferred for the accurate determination of pesticides to overcome the above difficulties encountered in LC/MS-based quantitative analysis of food samples. This technique is of particular interest since it corrects the recoveries of target analytes during sample clean-up and calibrates the instrumental biases [35, 36].

Thus, in this study, an isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) method for the accurate determination of carbaryl, carbendazim and carbofuran in vegetables was developed. These target pesticides were selected due to most frequently detected carbamate pesticides in the list of non-conforming pesticides among distributed agricultural products in Korea. In this study, conditions for separation, clean-up procedures and LC/MS/MS were optimized for accurate determination and then the method was validated. In addition, homogeneous Korean cabbage samples were prepared for testing the repeatability and reproducibility of the developed method. There are not a commercially available CRM with three target pesticides, but ERM-BC403 cucumber sample has a certified value of carbendazim. Thus, the developed ID-LC/MS/MS method was validated by analyzing of the value of carbendazim in ERM-BC403 cucumber sample. Finally, to confirm the feasibility of the method, the levels of three pesticides were analyzed in various vegetables, and ginseng from local markets in Korea.

Materials and Methods

Chemicals and Reagents

Carbaryl, carbendazim and carbofuran were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purities of these materials were determined in this laboratory (LC/UV analysis for structure-related impurities, Karl-Fisher titration for water content, and thermogravimetric analysis for non-volatile impurities) and they were subsequently used without further purification. A detailed description of the mass balance method for purity assessment can be found elsewhere [37, 38]. The purity of carbaryl was (99.15 ± 0.20) %, that of carbendazim was (99.85 ± 0.26) %, and that of carbofuran was (99.70 ± 0.19) %. Isotope analogues, $^{13}\text{C}_6$ -carbaryl (99%), and $^{13}\text{C}_6$ -carbofuran (99%), were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA), while carbendazim- d_4 (99.5%) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Ammonium formate and formic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade organic solvents, methanol and acetonitrile, were obtained from Burdick and Jackson (Muskegon, MI, USA).

Standard Solutions

A stock solution containing approximately 2 mg/kg of carbaryl, carbendazim, and carbofuran was gravimetrically prepared in 5 mM aqueous ammonium formate solution and methanol mixture (1:1, v/v) and stored at -20 °C. A working solution was prepared immediately prior to use with same solvent of stock solution. An isotope standard solution of $^{13}\text{C}_6$ -carbaryl, carbendazim- d_4 and $^{13}\text{C}_6$ -carbofuran was prepared in a similar way. An isotope ratio standard solution was prepared gravimetrically by mixing the standard solution and isotope standard solutions in an isotopic ratio of 1:1.

Sample Preparation and Clean-Up

Korean cabbages were purchased from a local cabbage farm and ginseng was purchased from a local ginseng farm in Korea. They were freeze-dried and pulverized to give powders (50–250 m powder size) for use as reference materials later. Water content of Korean cabbage and ginseng was measured before and after freeze-drying and the values were 97.5% and 85.8%, respectively; thus, 1 g of powder corresponded to approximately 20–40 g of raw Korean cabbage and 10–20 g of ginseng. Carrots, cabbages, and cucumbers were purchased from a local market in Korea and were minced using a mechanical grinder.

For sample preparation, 20 g of each minced raw vegetables or 1 g of Korean cabbage powder or ginseng powder was employed. These samples were weighed into a 50-mL conical tube and spiked with the isotope standard solution. An appropriate amount of the carbamate pesticide stock solution was fortified to the samples for the method validation before spiking the isotope solution. In the case of the powdered samples (Korean cabbage or ginseng), the isotope standard solution was spiked into the sample followed by 10 mL of water to reconstitute as the raw material shape. The extraction of carbamate pesticides was achieved by the addition of 20 mL acetonitrile to the sample and mixing with a mechanical shaker for 10 min. The sample was then centrifuged at 3900 rpm (1533 rcf) for 5 min and the supernatant was transferred into a new tube. Subsequently, 3 g of NaCl was added to the extract, shaken for 5 min, centrifuged at 3900 rpm (1533 rcf) for 5 min to separate the acetonitrile and water, and then the upper layer (acetonitrile) was transferred into a new tube. The extract (acetonitrile layer) was dried under N₂ gas. The residue reconstitution solvent was the mixture of methanol and dichloromethane (1:99, v/v) for the NH₂ cartridge. In the case of the ENVI-Carb/LC-NH₂ cartridge, the collected supernatant (acetonitrile layer) was added with toluene in a 3:1 ratio. The eluent from each SPE cartridge was evaporated under N₂ gas and reconstituted with 5 mM aqueous ammonium formate solution and methanol mixture (1:1, v/v).

LC/MS/MS Analysis

Separation was accomplished using the Agilent 1200 series LC system from Agilent Technologies (Palo Alto, CA, USA). 10 µL of an aliquot of the sample was injected onto a Waters Symmetry Shield C18 (2.1 mm i.d. × 150 mm, 3 µm) column with an Atlantis C18 guard column. The mobile phases consisted of a 5 mM aqueous ammonium formate solution with 0.1% formic acid as solvent A and acetonitrile as solvent B. Separation was achieved by gradient elution as follows; starting with 100% solvent A for 3 min, following by change to 50% solvent A for 15 min and then back to 100% solvent A for 7 min for next run. The flow rate was 0.2 mL/min. This LC system was interfaced with an API 2000 mass spectrometer from Applied Biosystems (Foster City, CA, USA). The mass spectrometry was operated in a positive mode with spray voltage of 4300 V. For MS/MS analysis, the selected reaction monitoring (SRM) mode was employed. The collision energy for the SRM mode was set to 9 V for carbaryl, 20 V for carbendazim and 30 V for carbofuran. The chemical structures and their product ion mass spectra are shown in Fig. 1.

Calculations and Measurement Uncertainty

The mass fraction of carbaryl, carbendazim and carbofuran (C_{sample}) in food samples were determined from the ID-LC/MS/MS analysis, as follow:

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

where C_{sample} is the mass fraction of the analyte (carbamate pesticides); $M_{\text{is-sol,spiked}}$ corresponds to the mass the isotope-labeled analyte added to the sample; AR_{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, added to the isotope ratio standard solution; $C_{\text{s-sol}}$ is the concentration of the analyte in the standard solution; M_{sample} is the mass of the sample, $AR_{\text{std.mix.}}$ corresponds to the observed area ratio of the analyte and the isotope-labeled analyte from the LC/MS/MS analysis of the isotope ratio standard solution. The uncertainty of the measurement values was evaluated according to the protocol described in previous studies [39–41] which follows the Guide to the Expression of Uncertainty in Measurements [42].

Results and Discussion

ID-LC/MS/MS Analysis

Full-scan mass spectra of target analytes were observed by direct infusion. All target analytes were ionized to their protonated forms $[M + H]^+$ at m/z 202 for carbaryl, m/z 192 for carbendazim, and m/z 222 for carbofuran in positive ion mode. Collisionally induced dissociation (CID) mass spectra were observed in product ion mode. The CID mass spectra (Fig. 1) of carbaryl and carbofuran were dominated by fragment ions at m/z 145 and m/z 165, which were attributed to $[M + H - \text{CONCH}_3]^+$. For carbendazim, the dominant fragment ion at m/z 160 was attributed to $[M + H - \text{CH}_3\text{OH}]^+$. The SRM channels were selected at m/z 202 → 145 for carbaryl, m/z 208 → 151 for ¹³C₆-carbaryl, m/z 192 → 160 for carbendazim, m/z 196 → 164 for carbendazim-*d*₄, m/z 222 → 165 for carbofuran, and m/z 228 → 171 for ¹³C₆-carbofuran. Figure 2 shows a comparison of the LC/MS chromatogram (Fig. 2a, b, selected ion monitoring (SIM) mode) and the LC/MS/MS chromatogram (Fig. 2c, d, selected reaction monitoring (SRM) mode) for the extracts obtained from fortified Korean cabbage and cleaned-up by the ENVI-Carb/LC-NH₂ cartridge. Although the sample was analyzed after

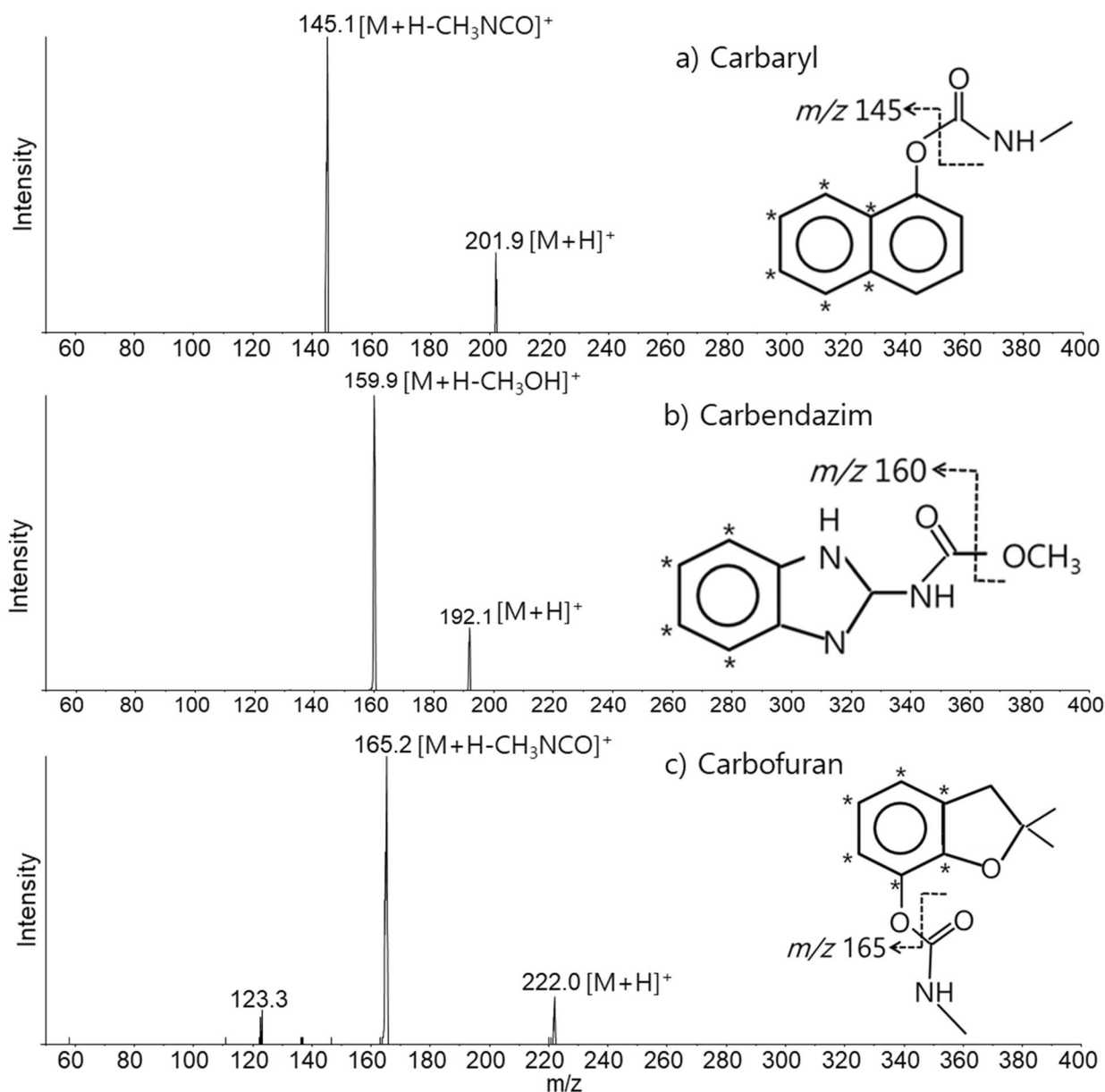


Fig. 1 Chemical structures and product ion mass spectra of carbaryl, carbendazim and carbofuran with collision induced dissociation (CID) pattern. * are indicating the position of ^{13}C or d_4 for isotope analogues

the clean-up step, significant co-eluting matrix materials were still detected in SIM mode Fig. 2a, b, thereby indicating the ability of the SRM mode to effectively select the analyte ions.

Selection of the SPE Cartridge

An appropriate sample clean-up step is necessary in sample preparation process for the food analysis. After testing recoveries of the target pesticides following the SPE cartridge and matrix interference profiles [43, 44], an NH₂ cartridge was initially selected due to its proper recovery and

matrix interference profile (data not shown). However, it was unable to remove color effectively from the vegetables samples, thereby, negatively affecting the column efficiency and easily clogging a guard column. Finally, the ENVI-Carb/LC-NH₂ cartridge was adopted for the sample preparation procedure as it was most effective in color removal.

Method Validation

The LC/MS/MS conditions and the sample clean-up procedure for the analysis of three target carbamate pesticides in Korean cabbage were evaluated. Korean cabbage

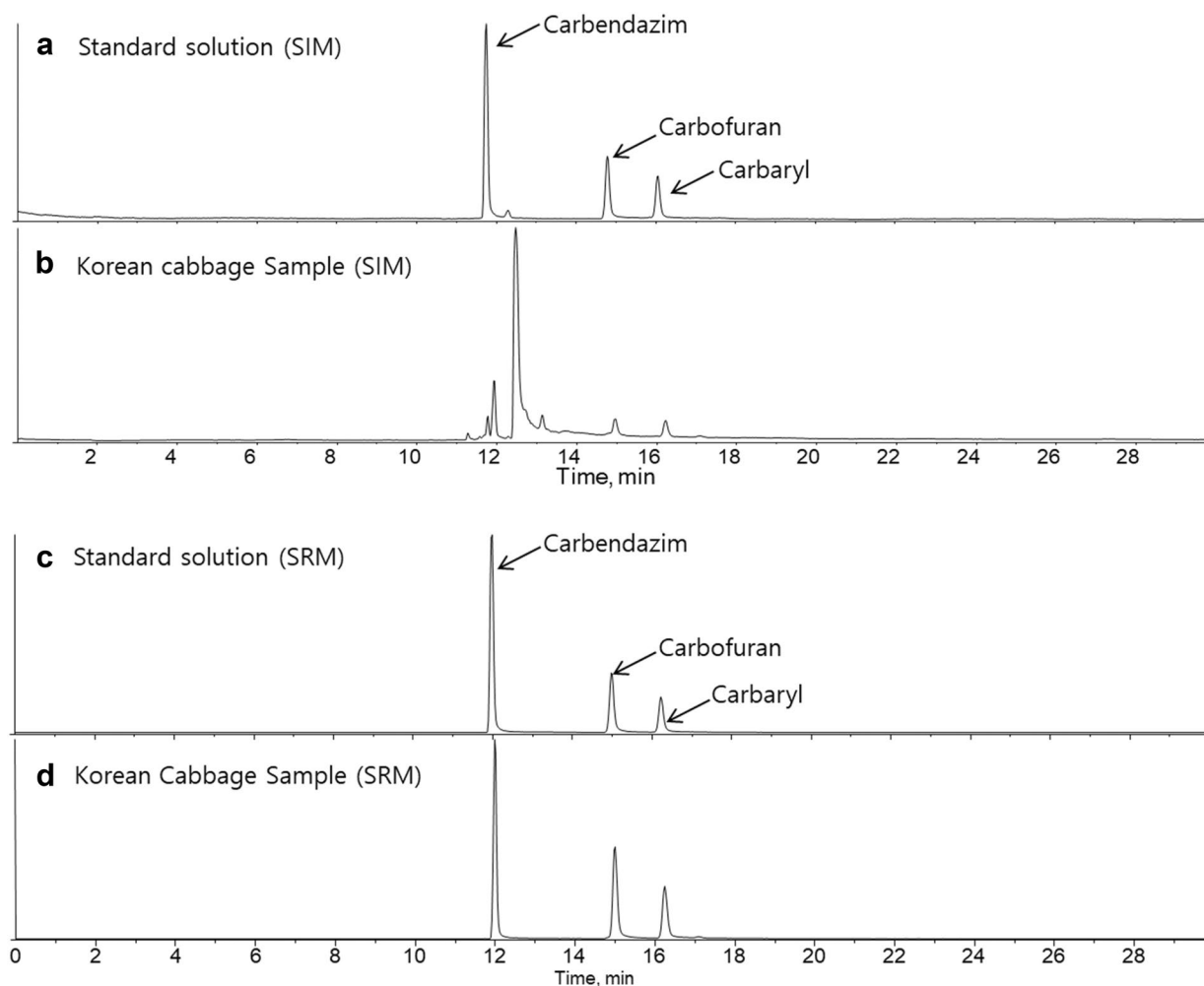


Fig. 2 Total ion chromatograms (TICs) of carbaryl, carbendazim and carbofuran with SIM mode (**a**, **b**) and SRM mode (**c**, **d**) by LC/MS/MS. TICs of **a** and **c** are for standard solution and TICs of **b** and **d** are for Korean cabbage sample after SPE procedure

powder samples were gravimetrically fortified with 1 mg/kg mixture solution of three target analytes and appropriate amount of their isotope standard solutions. The ratios of the fortified level to the measured level were observed from four sub-samplings. Table 1 lists the ID-LC/MS/MS results obtained for carbaryl, carbendazim, and carbofuran. The measured values agreed with the fortified values within their uncertainties and the ratios of the fortified level to the measured level for three target analytes ranged from 98 to 102%. For evaluation of repeatability and reproducibility of the method, homogeneous and well-defined CRM samples are necessary, but no commercial food CRM containing three target pesticides is available. Only carbendazim (ERM-BC406) in cucumber is available and its result will be shown later. Therefore, a homogeneous sample containing three target pesticides was prepared in this laboratory which has experiences for production of (C)RMs as an NMI of Korea. The homogeneous sample was prepared by fortifying

three target pesticides aqueous mixture solution to Korean cabbage powder which were described in “Materials and Methods” section. The Korean cabbage powder type transferred as a paste type. After homogenizing with mixer, the paste of Korean cabbage powder and three target pesticides mixture was freeze-dried and pulverized again producing powder type. Analysis of three sub-samplings from these homogeneous Korean cabbage powder samples was carried out for repeatability and the same test was performed on the other period for reproducibility. Table 2 shows the results of carbaryl, carbendazim and carbofuran by the developed ID-LC/MS/MS method. The relative standard deviation of three subsampling on each period was around 2% and the relative standard deviation of inter period was around 1%.

Considering the European MRLs for these three analytes set by the European Commission [45] and application of positive list system for agricultural products in Korea, the lower level, 0.05 mg/kg of the target analytes was fortified into various

Table 1 Measurement results of gravimetrically fortified samples to Korean cabbage powder by the developed ID-LC/MS/MS method

Analytes	Fortified value (mg/kg)	Measured value (mg/kg) ^a	Ratio (%) ^b
Carbaryl	#1 0.947	0.928 ± 0.033	98.0
	#2 0.970	0.968 ± 0.025	99.8
	#3 0.971	0.964 ± 0.031	99.3
	#4 0.915	0.923 ± 0.033	100.9
Carbendazim	#1 0.952	0.959 ± 0.031	100.7
	#2 0.975	0.993 ± 0.019	101.9
	#3 0.975	0.976 ± 0.028	100.1
	#4 0.919	0.918 ± 0.037	99.9
Carbofuran	#1 0.948	0.968 ± 0.027	101.7
	#2 0.971	0.963 ± 0.028	99.2
	#3 0.971	0.974 ± 0.028	100.2
	#4 0.916	0.900 ± 0.033	98.3

^aThe number after ± is the expanded uncertainty of the preceding value with the level of confidence of 95%, that is obtained combining relative standard deviations of multiple measurement for isotope ratio standard solution and for samples

^bRelative difference of the measured value from the gravimetrically fortified value

vegetables including cabbage, cucumber, and carrot obtained from local markets and homogenized by grinding. Blank test for these vegetable samples were carried out by the developed ID-LC/MS/MS method resulting in no peaks of three target analytes and if any, below the detection limit from food samples. Results of the ratios of fortified values to the measured values are listed in Table 3. The measured values agreed

with the fortified ones and all ratios ranged 98~102%. These results, therefore, confirmed that the developed ID-LC/MS/MS method could be reliable for the determination of three target analytes in the various vegetable samples, in addition to being acceptable at the lower level of the target analytes.

As mentioned above in this paragraph, ERM cucumber sample with carbendazim is available in commercial. The results by the developed ID-LC/MS/MS is illustrated in Fig. 3. The measured value of carbendazim in ERM-BC403 was (0.0724 ± 0.0013) mg/kg and the certified value is (0.074 ± 0.004) mg/kg. The measured value was obtained with four subsampling after following the attached protocols for reconstitution of cucumber. The expanded uncertainty of measured value was calculated combining systematic and random effects. The systematic uncertainty was composed with uncertainty for preparation standard solutions and relative standard deviations of multiple measurements for isotope ratio standard solutions. The random effect was the relative standard deviation of mass fractions of four subsamplings from ERM-BC403 sample. The measured value agreed with the certified value in their uncertainties; however, the uncertainty by the developed ID-LC/MS/MS method (1.8%) was much smaller than that of certification (5.4%) indicating that the method provides accurate measurement values.

The limits of detection (LODs) (signal to noise ratio of 3) of carbaryl, carbendazim, and carbofuran were 0.002, 0.001, and 0.001 mg/kg, respectively. The limits of quantitation (LOQs) were estimated by fortifying the Korean cabbage powder samples with each analyte at series of levels in the range of 0.005~0.010 mg/kg. LOQs (signal to noise ratios of 10) of carbaryl, carbendazim, and carbofuran were 0.008, 0.004 and 0.006 mg/kg, respectively. These LODs and LOQs of carbaryl,

Table 2 Repeatability and reproducibility test results by the developed ID-LC/MS/MS method by measuring a homogenized Korean cabbage sample

		Results by ID-LC/MS/MS (mg/kg)			
		Sample no	Carbaryl	Carbendazim	Carbofuran
Period 1	Subsample 1		2.984 ± 0.004 ^a	2.967 ± 0.026	2.867 ± 0.020
	Subsample 2		2.985 ± 0.020	3.012 ± 0.013	2.898 ± 0.024
	Subsample 3		2.992 ± 0.021	3.013 ± 0.013	2.903 ± 0.027
	Average		2.987	2.997	2.889
	Standard deviation		0.004 (0.14%)	0.026 (0.88%)	0.020 (0.68%)
Period 2	Subsample 1		3.067 ± 0.018	2.88 ± 0.029	2.94 ± 0.039
	Subsample 2		2.992 ± 0.050	2.92 ± 0.039	2.93 ± 0.055
	Subsample 3		3.091 ± 0.056	3.02 ± 0.045	2.93 ± 0.036
	Average		3.050	2.938	2.934
	Standard deviation		0.052 (1.7%)	0.073 (2.5%)	0.004 (0.13%)
	Average between periods		3.018	2.967	2.911
	Standard deviation between periods (%)		0.045 (1.48%)	0.042 (1.41%)	0.032 (1.09%)

^a The number after ± is the expanded uncertainty of the preceding value with the level of confidence of 95%, that is obtained combining relative standard deviations of multiple measurement for isotope ratio standard solution and for samples

Table 3 Measurement results of gravimetrically fortified samples to various vegetables by ID-LC/MS/MS

Analytes		Fortified value (mg/kg)	Measured value (mg/kg) ^a	Ratio (%) ^b
Cabbage	Carbaryl	0.036	0.035 ± 0.002	98.4
		0.036	0.036 ± 0.002	98.4
	Carbendazim	0.037	0.038 ± 0.003	101.0
		0.037	0.038 ± 0.003	101.1
Cucumber	Carbaryl	0.045	0.045 ± 0.004	99.2
		0.045	0.044 ± 0.004	98.1
	Carbendazim	0.049	0.0487 ± 0.001	99.9
		0.049	0.0492 ± 0.001	100.9
Carrot	Carbaryl	0.050	0.0508 ± 0.003	101.0
		0.050	0.0520 ± 0.002	103.5
	Carbofuran	0.061	0.0615 ± 0.001	100.7
		0.061	0.0612 ± 0.001	100.2
Cucumber	Carbaryl	0.048	0.0483 ± 0.001	101.3
		0.048	0.0497 ± 0.001	102.8
	Carbendazim	0.049	0.0505 ± 0.001	102.8
		0.050	0.0510 ± 0.001	102.3
Carbofuran	0.060	0.0608 ± 0.001	101.7	
	0.061	0.0616 ± 0.001	101.6	

^aThe number after ± is the expanded uncertainty of the preceding value with the level of confidence of 95%, that is obtained combining relative standard deviations of multiple measurement for isotope ratio standard solution and for samples

^bRelative difference of the measured value from the gravimetrically fortified value

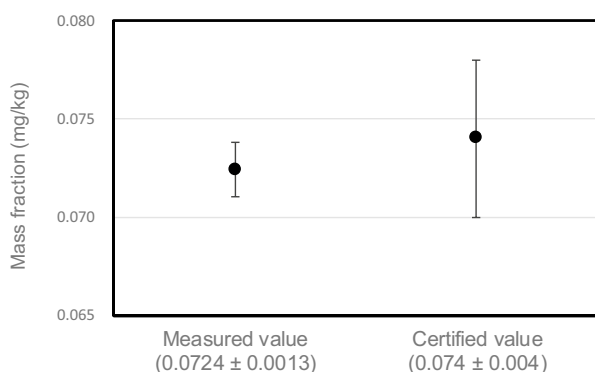


Fig. 3 Result of carbendazim in ERM-BC403 cucumber sample by the developed ID-LC/MS/MS method. The error bars represent the expanded uncertainty of measured value with the 95% confidence level

carbendazim, and carbofuran were lower than the MRLs established by the EU and by many other economies.

Application of the ID-LC/MS/MS Method to Various Vegetables and Fruits

Following validation of the ID-LC/MS/MS method, it was applied for the determination of three target analytes in commercial vegetable samples purchased from markets in Korea. Korean cabbage powder sample and the ginseng powder

sample in Table 4 were batches previously prepared in 2009. Table 4 summarizes the results obtained for each analyte with the Korean and EU MRLs for each vegetable. Although no signals corresponding to carbaryl and carbofuran was obtained from all selected samples, signals corresponding to carbendazim were observed for carrot, Korean cabbage and ginseng. The carbendazim signals were below the LOQs for the majority of samples. According to the report from the Seoul Research Institute of Public Health and Environment and the Ministry of Food and Drug Safety of Korea, the frequency of detection for carbendazim and carbaryl in various agricultural food products and fruits has increased since 2006 in Korea [46].

Conclusions

An isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS) method for the accurate analysis of carbamates pesticides, carbaryl, carbendazim, and carbofuran in vegetables was established. To ensure the removal of the matrix interferences from these food samples, an ENVI-Carb/LC-NH₂ solid-phase extraction cartridge was employed in the sample clean-up procedure. For method validation, three target pesticides were fortified into the Korean cabbage powder, carrot, cabbage, and cucumber samples. All values of the target pesticides determined by the developed ID-LC/MS/MS method agreed with the gravimetrically fortified values within

Table 4 Determination of carbaryl, carbendazim, and carbofuran in various commercial fruits and vegetables from a Korean market using ID-LC/MS/MS and maximum residue limits (MRLs) of pesticides in Korea and EU^a

Compound	(mg/kg)		Korean cabbage	Cabbage	Cucumber	Carrot ^a	Ginseng ^b
Carbaryl	MRL	Kr	0.5	0.5	0.5	–	–
		EU	0.01	0.01	0.01	0.01	0.05
	Measured value		N/D	N/D	N/D	N/D	N/D
Carbendazim	MRL	Kr	0.7	1.0	1.0	0.2	0.5
		EU	0.1	0.1	0.1	0.1	0.1
	Measured value		0.90 ± 0.11 ^c	N/D	N/D	(0.006) ^d	(0.01) ^d
Carbofuran	MRL	Kr	0.05	0.5	0.05	0.05	0.2
		EU	0.002	0.002	0.002	0.002	0.05
	Measured value		N/D	N/D	N/D	N/D	N/D

^aReferences from www.foodsafetykorea.go.kr for Korea MRLs and www.ec.europa.eu for EU MRLs

^bThe MRLs for ginseng are not established in Korea, therefore, the MRLs in table correspond to red ginseng

^cThe result values of Korean cabbage and ginseng were determined with one gram of powder

^dThe values in parenthesis were determined based on the detected signals but the values were below the limit of quantitation, thus uncertainties were not calculated

their uncertainties. Homogeneous Korean cabbage power samples containing three target pesticides were prepared for tests of repeatability and reproducibility of the method. The repeatability and the reproducibility of the method was below 2%. It proved that this method was reliable for the accurate analysis of three target pesticides in food samples. The feasibility of this method was tested with various vegetables and ginseng purchased from a local market in Korea. Although carbaryl and carbofuran were not detected in purchased food products, carbendazim was observed in the carrot, Korean cabbage, and ginseng samples below the limit of quantitation. This method will be applied for the certification of carbaryl, carbendazim and carbaryl in reference materials by KRISS later.

Acknowledgements This work was supported by the Korea Research Institute of Standards and Science under the project ‘Establishment of national standard system in organic analysis area’, Grand no.17011053.

Compliance with Ethical Standards

Conflict of Interest The authors Seonghee Ahn, Ji-Young Lee, and Byungjoo Kim declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent not applicable.

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