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ARTICLE

Development of cabbage reference material for multi-residue pesticide analysis

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Abstract Cabbage reference material for pesticide multi-residue analysis was developed in accordance with the ISO Guide 35, ISO Guide 13528 and European Union Reference Laboratories-Proficiency Test standard protocols. Ten pesticides (acetamiprid, azoxystrobin, boscalid, buprofezin, carbendazim, difenoconazole, ethofenprox, imidacloprid, pyraclostrobin and tebuconazole) detected at relatively high levels in agricultural products in Korea were selected for this study. The developed material was evaluated for homogeneity and stability according to the statistical assessment method specified by international standards. Analysis of variance was carried out to calculate the within-bottle standard variation (s_{wb}) and the between-bottle standard variation (s_{bb}). Values of s_{wb} and s_{bb} varied by less than 4.7% of assigned values. Homogeneity was also assessed using Cochrane testing of outliers. All pesticides in the material were uniformly distributed within or between all bottles. Stability tests were conducted at room temperature (20–30 °C) for 12 days, under cold conditions (4–8 °C) for 40 days, under freezing conditions (− 20 °C) for 70 days and under deep freezer conditions (− 80 °C) for 234 days. Stability was evaluated based on the ISO Guide 35 statistical model, and results showed no significant decrease in stability during storage for any pesticide under any condition. We therefore conclude that the cabbage material could be used for future proficiency tests and/or validation of pesticide residue analysis.

Keywords Cabbage · Multi-residue pesticide analysis · Proficiency test · Reference material · Stability

Introduction

Many pesticides are widely used to protect agricultural crops and products from insects and diseases. However, if residual pesticides remain at levels above the maximum residue limits, they may have adverse effects on human health [1–3]. In addition, since residue analysis data determined from various instruments are used in exposure assessment by health and safety authorities, accurate residue level determination is paramount [4, 5]. Thus, it is important to monitor the reliability of analytical results, hence the introduction of certified reference materials (CRMs) as tools for assessing the quality of measurements [6]. Although some CRMs have been developed for environmental organic pollutants such as organochlorine pesticides and polychlorinated biphenyls [7], and for vegetable or fruit matrices such as brown rice [8], cucumber [6], soybean [3] and apples [9], CRMs covering the vast number of agricultural product matrices have not yet been developed [6].

Within Europe, in order to harmonise regulatory control at the EU level, laboratories are required to use validated methods and to demonstrate satisfactory performance by regular participation in proficiency testing (PT) [5]. For European Union Proficiency Tests (EUPTs), ~ 150 official laboratories for pesticide residue testing have been approved to conduct tests and ensure the quality, accuracy

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and comparability of results based on their different capabilities and skills [10]. For proficiency tests in Europe, EUPT samples containing multi-residue pesticides suitable for homogeneity and stability testing are prepared based on the IUPAC/AOAC protocol [10] and sent to participating laboratories to monitor and evaluate the analytical accuracy and report the results. Over a 12-year period, more than 10 tests have reported on over 10 different fruit and vegetable matrices [10].

In Korea, several reference materials have been developed, including seven trace metal elements (Pb, Cd, Cr, Cu, Zn, Mn and Fe) in wastewater [11] and phthalate plasticisers (DMP, DEP, DBP, BBP, DEHP and DnOP) in acrylonitrile–butadiene–styrene resin [12] related to environmental organic pollutants, as well as four pesticides (diazinon, chlorpyrifos, α-endosulfan and β-endosulfan) in Chinese cabbage [13]. Since many authorised laboratories conducted pesticide residue analysis using simultaneous analysis of multi-residue pesticides in agricultural products, several reference materials have been developed as tools for PT, assessing the reliability and performance of analytical methods by using green pepper material (bifenthrin, chlorfenapyr, chlorpyrifos, lambda-cyhalothrin, fenitrothion, fenpropathrin, iprobenfos, isoprothiolane, kresoxim-methyl and procymidone) [14] and tomato material (acetamiprid, buprofezin, chlorfenapyr, clothianidin, diethofencarb, ethofenprox, fenamidone, iprodione, novaluron, procymidone, pyraclostrobin, pyridaben, pyrimethanil, pyriproxyfen, spiromesifen, tebuconazole, tebufenozide, tetraconazole, thiamethoxam and triflumizole) [15]. However, since various matrices and pesticides are included in the analysis of residual pesticides, the development of numerous reference materials based on international guidance is required.

In the present study, we developed cabbage reference material containing 10 pesticides detected at relatively high levels in agricultural products in Korea to provide useful information on the production of a reference material and to use as a crucial tool for the proficiency test. Prior to preparation, analytical method validation was conducted to ensure the appropriate method for each pesticide. Homogeneity, short- and long-term stability studies were also performed and monitored in accordance with ISO Guides and the European Union Reference Laboratories-Proficiency Test (EURL-PT) protocol to evaluate transformation and storage.

Materials and methods

Standard substances and reagents

Ten pesticides (acetamiprid, azoxystrobin, boscalid, buprofezin, carbendazim, difenoconazole, ethofenprox, imidacloprid, pyraclostrobin and tebuconazole) detected at relatively high levels in agricultural products in South Korea were purchased from Sigma-Aldrich (Steinheim, Germany) and applied to cabbage reference material. HPLC-grade acetonitrile was purchased from Burdick & Jackson (Muskegon, MI, USA). Diphosphorus dioxide (P_2O_2) was purchased from Sigma-Aldrich. A QuEChERS extraction kit (roQ) and a dispersive solid phase extraction kit (roQ) were purchased from Phenomenex (USA).

Preparation of reference material

Organic certification-grade cabbage was purchased from a local market, chopped, pulverised and freeze-dried at a temperature (− 130 °C) for 6 days. To make a fine powder, material was ground using a JL-1000 grinder (Hibell, Seoul, Korea) and stored under freezing conditions (below − 20 °C) until use. An aliquot (0.1 mL) of each pesticide solution (1000 μg/mL) dissolved in acetonitrile was used to treat cabbage powder (1 kg) to obtain the 0.1 mg/kg sample for each pesticide, to which 2 L of distilled water was added and mixed at 250 rpm for 1 h using a table mixer (KMM 760, KENWOOD, Seoul, Korea). Samples were subsequently stored in a deep freezer (MDF-U54V, SANYO, Osaka, Japan) at − 80 °C for 1 day and freeze-dried at a temperature (− 130 °C) using a chemical-free freeze dryer (FDCF-12006, OPERON, Seoul, Korea) for 6 days. Samples were ground using a grinder for ∼ 10 min and mixed using a table mixer for 30 min to improve homogeneity. A portion (100 g) was placed in each of ten brown glass bottles (300 mL) and stored in a deep freezer at − 80 °C until use.

Analytical conditions

All samples were analysed using liquid chromatography coupled to a 6460 triple quadrupole tandem mass spectrometry (LC–MS/MS) (Agilent, Santa Clara, CA, USA), with MassHunter Workstation software version B.06.00 Build 6.0.6025.4 SP4, equipped with a 1260 infinity HPLC (Agilent). A C18 ZORBAX Eclipse Plus RRHD column (1.8 μm, 2.1 × 100 mm; Agilent) was used for separation with the gradient elution method using 0.1% formic acid/5 mM ammonium formate in water (buffer A) and 0.1% formic acid/5 mM ammonium formate in methanol (buffer B). The initial mobile phase of 95:5 A:B (v/v) was held for

0.5 min, then changed to 50:50 A:B (v/v) over 3.5 min and then to 0:100 A:B (v/v) over 17.0 min, held at this for 3.0 min, and finally changed to 95:5 A:B (v/v) over 25 min. The injection volume and flow rate were 1.0 μL and 0.2 mL/min, respectively. The column temperature was maintained at 35 °C throughout sample analysis. MS/MS was operated in positive electrospray ionisation and multiple reaction monitoring modes. The gas temperature was 300 °C, and the capillary voltage was 4000 V. Liquid nitrogen gas was used as the nebuliser gas (35 psi). All other information is presented in Table 1.

Establishment of a method for validation of the 10 pesticides in cabbage reference material

Control cabbage reference material samples not containing the 10 pesticides (5 g) were treated with pesticides to give a final pesticide concentration of 0.1 mg/kg, 10 mL of deionised water was added, and the samples were allowed to settle for 1 h. An additional 10 mL of acetonitrile was added and shaken vigorously using a ceramic homogeniser for 2 min. A QuEChERS extraction kit containing 4 g of $MgSO_4$, 1 g of NaCl, 1 g of sodium tribasic dihydrate and 0.5 g of sodium dibasic sesquihydrate was added and centrifuged at 3000 rpm for 5 min. The supernatant was filtered with a 2-μm syringe filter, and 1 mL of filtrate was diluted twofold with 1 mL of acetonitrile. Aliquots (200 μL) were removed and mixed with 750 μL of water and 50 μL of acetonitrile for construction of a matrix matched calibration curve, and final samples were analysed by LC–MS/MS.

Calibration curves

Matrix matched calibration curves were constructed using control samples to compensate for matric effect. Approximately 10 mg of each standard pesticide was dissolved in 10 mL of acetonitrile to obtain a 1000 mg/L stock solution, from which 1 mL was added to a 50-mL volumetric flask and made up to 50 mL with acetonitrile. This mixture solution was diluted with acetonitrile to make each standard solution. For matrix effects, final concentrations were diluted with acetonitrile, water and control sample extract (without pesticides) to 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 μg/L to construct the calibration curve. All calibration solutions contained 200 μL of matrix from control sample extract, 750 μL of water and 50 μL of acetonitrile.

Homogeneity

Target pesticides should be uniformly distributed within or between all bottles. 5 g of aliquots was removed from the bottom, and then middle and top of each of the 10 bottles in order to check homogeneity were analysed by LC–MS/MS. Homogeneity was assessed in accordance with ISO Guide 35 [16], ISO Guide 13528 [17] and the EURL-PT protocol,

Table 1 LC–MS/MS information for the 10 tested pesticides

Pesticide	Precursor Ion (m/s)	Product Ion (m/s)	Fragment Voltage (V)	Collision energy (CE, V)
Acetamiprid	223.1	126.1	90	10
		56.1		10
Azoxystrobin	404.2	372.1	100	6
		344.1		22
Boscalid	343.1	307.1	100	20
		140.0		20
Buprofezin	306.2	201.1	90	10
		116.0		8
Carbendazim	192.1	160.1	90	12
		132.0		30
Difenoconazole	405.9	336.9	135	14
		251.0		18
Ethofenprox	394.1	177.1	50	6
		135.0		20
Imidacloprid	256.1	209.1	60	8
		175.1		20
Pyraclostrobin	388.1	194.1	90	2
		163.0		22
Tebuconazole	308.2	125.0	110	30
		70.0		16

which were used for statistical evaluation based on the IUPAC/AOAC Harmonized Protocol for Proficiency Testing [18]. Standard deviations between bottles (s_{bb}) and within bottles (s_{wb}) were calculated using Eqs. (1) and (2) in accordance with ISO Guide 35 [3, 16] as follows:

$$S_{bb} = \sqrt{\frac{MS_{among} - MS_{within}}{n}} \quad (1)$$

$$S_{wb} = \sqrt{MS_{within}} \quad (2)$$

where MS_{among} and MS_{within} represent the mean squares between groups and within a group, respectively, and n represents the number of measurements per bottle [3, 16].

ISO Guide 13528 [17] suggests using Eqs. 3–5 to determine values of s_w and s_s as follows:

$$S_x = \sqrt{\sum (X_t.. - \bar{X})^2/(g-1)} \quad (3)$$

$$S_w = \sqrt{\sum w_t^2/(2g)} \quad (4)$$

$$S_b = \sqrt{S_x^2 - (S_w^2/2g)} \quad (5)$$

where s_x represents the standard deviation of the average sample analysed, t represents the sample ($t = 1, 2, 3,\ldots$ g), X and $\bar{X}$ represent the data analysed and the average data, respectively, and s_w and s_b represent the within-samples standard deviation and between-samples standard deviation, respectively. A value of s_b less than 0.3σ indicates acceptable homogeneity, where σ is the target standard deviation representing a relative standard deviation (RSD) of 25% multiplied by the analytical values [17].

Finally, EURL-PT suggests using Eqs. (6) and (7) to determine s_b^2 and the constant c as follows [5, 10]:

$$S_b^2 = S_x^2 - \left(\frac{S_w^2}{2}\right) \quad (6)$$

$$c = F_1(0.3\sigma)^2 + F_2 S_w^2 \quad (7)$$

Determination of assigned value and uncertainty

The assigned value was obtained from the average value calculated from 30 samples. The uncertainty due to possible inhomogeneity was assessed in accordance with ISO Guide 35 [16] that suggests using Eq. (8) to determine the value of uncertainty (u_{bb}) as follows:

$$u_{bb} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu MS_{within}}} \quad (8)$$

where n represents the number of measurements per bottle and νMS_{within} represents the number of degrees of freedom of MS_{within} [9]. When MS_{among} is larger than MS_{within}, the s_{bb} of Eq. (1) and u_{bb} of Eq. (8) can be applied, and two Eqs. (1) and (8) can be employed. Generally, the uncertainty due to inhomogeneity was treated using the larger of the values for either s_{bb} or u_{bb} to prevent overestimation [3]. On the other hand, if the MS_{among} is lower than MS_{within}, only Eq. (8) can be applied.

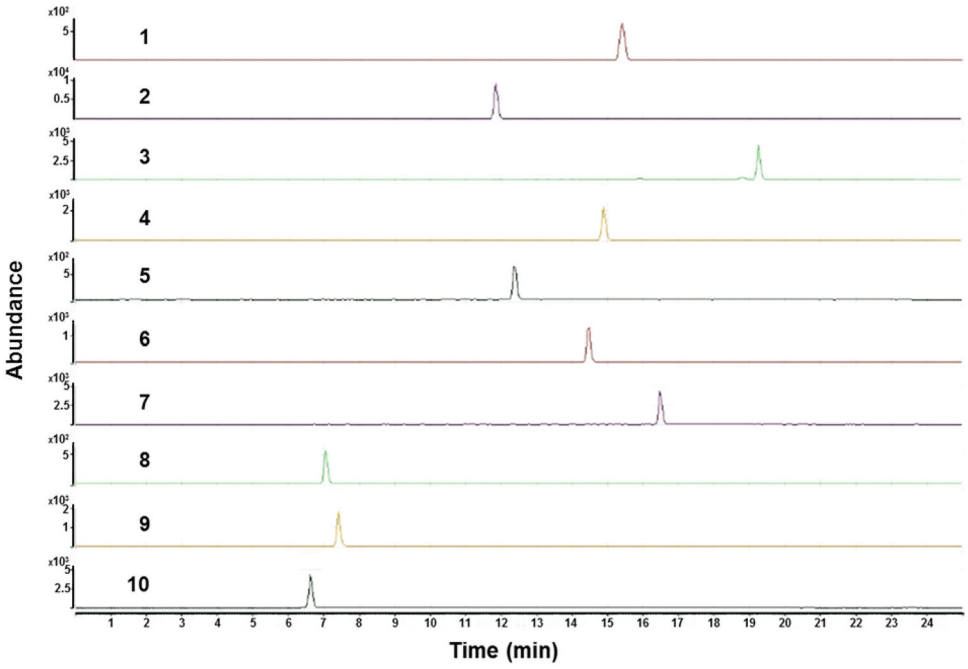

Fig. 1 LC–MS/MS chromatogram of the 10 pesticides spiked at 100 ng/g in cabbage samples. From top: acetamiprid (*1*), azoxystrobin (*2*), boscalid (*3*), buprofezin (*4*), carbendazim (*5*), difenoconazole (*6*), ethofenprox (*7*), imidacloprid (*8*), pyraclostrobin (*9*) and tebuconazole (*10*)

Determination of water content

An aliquot of 5 g was taken from cabbage reference material with a proven homogeneity and placed in a desiccator containing P_2O_2 for 7 days, after which it was weighted and the weight change was determined.

Stability of the 10 pesticides

Stability testing was carried out by incubating material for 12 days at room temperature (~ 20–30 °C) for 40 days under cold conditions (~ 4–8 °C), for 70 days under freezing conditions (− 20 °C) and for 234 days under deep freezer conditions (− 80 °C). Temperatures were monitored and evaluated in accordance with ISO Guide 35 [16]. The slope (b_1) and intercept (b_0) of the linear regression were calculated using Eqs. (9) and (10) as follows:

$$b_1 = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sum_{i=1}^{n}(X_i - \bar{X})^2} \quad (9)$$

$$b_0 = \bar{Y} - b_1\bar{X} \quad (10)$$

where X_i and Y_i represent the time and the concentration at time I, and $\bar{X}$ and $\bar{Y}$ represent the average of X_i and Y_i, respectively [3]. The value of $s(b_1)$ was calculated using Eq. (11), and s was calculated using Eq. (12) as follows:

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}} \quad (11)$$

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - b_0 - b_1X_i)^2}{n-2} \quad (12)$$

Results and Discussion

Method validation

The LC–MS/MS chromatograms of matrix samples indicated no interference for the 10 pesticides (Fig. 1). The method limit of quantification (MLOQ) ranged from 0.004 to 0.02 mg/kg, demonstrating the ability of LC–MS/MS to detect trace amounts of pesticides in cabbage reference material. Recoveries of each pesticide ranged from 93.0 ± 0.12 to 125.1 ± 0.01% for low and high pesticide levels, and the RSD was less than 0.37% for all pesticides. These values are low enough to confirm the precision and accuracy of the analytical method for determination of pesticide residues [19]. Additionally, the calibration curves gave values of at least 0.99943 for all pesticides, confirming that the analytical procedure is acceptable for the determination of assigned values, homogeneity and stability. The details are summarised in Table 2.

Homogeneity

Homogeneity is a very important factor because inhomogeneity can affect the results of PT and/or the validation of analytical methods. Assessment of homogeneity is very closely correlated with the evaluation of pesticide residue analysis. Using the assessment method specified in ISO Guide 13528 [17], all values of between-samples standard deviation (s_b) were less than 0.3σ (Table 3). Furthermore, using the assessment method stipulated in the EURL-PT protocol, analysis data were investigated for outliers using Cochrane testing. The results showed that the squares of between-samples standard deviation (s_b) were less than the constant 'c' (Table 4), indicating that all pesticides were distributed homogeneously in the cabbage reference material, demonstrating that acceptable homogeneity was achieved for all 10 pesticides.

Using statistical assessment outlined in ISO Guide 35 [16], the within-bottle standard deviation (s_{wb}) and the between-bottle standard deviation (s_{bb}) were calculated by one-way analysis of variance (ANOVA, $p < 0.05$) [6, 20]. Values of s_{wb} ranged from 2.1 to 4.7% of assigned values for all pesticides, and values of s_{bb} ranged from 0.4 to 4.7% of assigned values (Table 5) [3]. The results of statistical analysis revealed no statistical differences within and between bottles for each pesticide, and confirmed that the homogeneity was below the 10% level that is considered acceptable [6].

Assigned values and uncertainty

Assigned values were found to be similar to spiked levels. The values obtained by LC–MS/MS analysis of 30 replicates ranged from 0.092 to 0.113 mg/kg, and the RSD was less than 3.1% (Table 5) [3]. The uncertainty (u_{bb}) due to possible inhomogeneity that can be hidden by method repeatability was calculated in accordance with ISO Guide 35 [6, 16]. All values were less than 0.8% of assigned values, indicating that uncertainty did not affect determination of assigned values or the analysis proficiency test results.

Water content and stability

The water content of freeze-dried material was about 0.3%, which is low enough to be ignored when weighing, and low enough to inhibit the micro-degradation of target pesticides in materials stored at low temperatures. The stability of material containing pesticides was tested at room temperature, in cold, in freezing and in deep freeze conditions during transport and storage. The results were evaluated individually according to the statistical method specified in ISO Guide 35. The obtained RSD of the storage time was

Table 2 Validation of the method used for analysis of the 10 tested pesticides by LC–MS/MS

Pesticide	Fortified conc. (mg/kg)	Recovery[a] (%)	RSD[b] (%)	MLOQ[c] (mg/kg)	Linearity (r^2)
Acetamiprid	0.02	113.5 ± 0.05	0.04	0.001	0.99982
	0.1	106.5 ± 0.08	0.08		
Azoxystrobin	0.02	125.1 ± 0.01	0.01	0.002	0.99943
	0.1	107.1 ± 0.15	0.14		
Boscalid	0.02	101.0 ± 0.05	0.05	0.02	0.99993
	0.1	109.0 ± 0.23	0.21		
Buprofezin	0.02	120.3 ± 0.17	0.14	0.004	0.99965
	0.1	105.0 ± 0.23	0.22		
Carbendazim	0.02	97.6 ± 0.05	0.05	0.001	0.99972
	0.1	93.0 ± 0.12	0.13		
Difenoconazole	0.02	119.6 ± 0.03	0.03	0.02	0.99996
	0.1	104.1 ± 0.24	0.23		
Ethofenprox	0.02	113.5 ± 0.08	0.07	0.004	0.99996
	0.1	101.6 ± 0.07	0.07		
Imidacloprid	0.02	117.3 ± 0.04	0.03	0.01	0.99985
	0.1	105.8 ± 0.39	0.37		
Pyraclostrobin	0.02	116.2 ± 0.05	0.04	0.01	0.99992
	0.1	105.4 ± 0.08	0.08		
Tebuconazole	0.02	118.8 ± 0.20	0.17	0.02	0.99989
	0.1	108.2 ± 0.13	0.12		

[a]Average and standard deviation from triplicate experiments

[b]Relative standard deviation: (standard deviation/average) × 100

[c]Method limit of quantification was calculated as follows

[Limit of quantification for the instrument × final volume]/[injection volume × initial sample weight]

Table 3 Homogeneity results in accordance with ISO Guide 13528

Pesticide	ISO Guide 13528				
	s_x	s_w	s_b	0.3σ[a]	$s_b \leq 0.3\sigma$
Acetamiprid	3.7 × E−03	2.3 × E−03	3.3 × E−03	7.2 × E−03	Accept
Azoxystrobin	3.6 × E−03	2.5 × E−03	3.2 × E−03	8.4 × E−03	Accept
Boscalid	4.2 × E−03	3.6 × E−03	3.3 × E−03	8.2 × E−03	Accept
Buprofezin	5.0 × E−03	2.9 × E−03	4.5 × E−03	7.3 × E−03	Accept
Carbendazim	1.4 × E−03	9.9 × E−03	1.2 × E−03	6.9 × E−03	Accept
Difenoconazole	5.1 × E−03	5.8 × E−03	3.0 × E−03	8.1 × E−03	Accept
Ethofenprox	6.4 × E−03	4.2 × E−03	5.6 × E−03	7.8 × E−03	Accept
Imidacloprid	3.7 × E−03	3.7 × E−03	2.6 × E−03	8.2 × E−03	Accept
Pyraclostrobin	4.9 × E−03	2.8 × E−03	4.5 × E−03	8.2 × E−03	Accept
Tebuconazole	6.5 × E−03	3.0 × E−03	6.2 × E−03	8.4 × E−03	Accept

[a]σ represents the 25% relative standard deviation of analytical values

less than 6.2% at room temperature for 12 days, 6.2% in the cold for 40 days, 12.8 in freezing conditions for 70 days and 5.7% under deep freeze conditions for 234 days (Table 6). Likely, the absolute value of b_1 was less than the corresponding values of $t_{0.95,\ n-2\times s(b1)}$ ($t_{0.95,\ n-2} = 12.71$) in the ten pesticides. The result indicates no statistically significant decrease in the concentration of the pesticides [3]. Therefore, all pesticides in the cabbage reference material developed for multi-residue pesticide analysis were demonstrated to be stable when stored at the four different temperatures tested. Experimental details are summarised in Table 7.

Table 4 Homogeneity results in accordance with the EURL-PT protocol based on the IUPAC method

Pesticide	EURL-PT protocol (IUPAC method)				
	s_w^2	s_b^2	$(0.3\sigma)^2$	c	$s_b^2 < c$
Acetamiprid	5.2 × E−06	1.1 × E−05	5.2 × E−05	1.0 × E−04	Accept
Azoxystrobin	6.2 × E−06	1.0 × E−05	7.0 × E−05	1.4 × E−04	Accept
Boscalid	1.3 × E−05	1.1 × E−05	6.8 × E−05	1.4 × E−04	Accept
Buprofezin	8.5 × E−06	2.1 × E−05	5.4 × E−05	1.1 × E−04	Accept
Carbendazim	9.7 × E−07	1.4 × E−06	4.8 × E−05	9.1 × E−05	Accept
Difenoconazole	3.3 × E−05	9.0 × E−06	6.6 × E−05	1.6 × E−04	Accept
Ethofenprox	1.7 × E−05	3.2 × E−05	6.1 × E−05	1.3 × E−04	Accept
Imidacloprid	1.4 × E−05	6.6 × E−06	6.7 × E−05	1.4 × E−04	Accept
Pyraclostrobin	8.0 × E−06	2.0 × E−05	6.7 × E−05	1.3 × E−04	Accept
Tebuconazole	9.2 × E−06	3.8 × E−05	7.1 × E−05	1.4 × E−04	Accept

σ represents the 25% relative standard deviation of analytical values

Table 5 Results of assigned values, within-samples standard deviation (s_{wb}), between-samples standard deviation (s_{bb}) and uncertainty (u_{bb})

Pesticide	Assigned values[a] ± SD[b] (mg/kg)	RSD[c] (%)	ISO Guide 35 (mg/kg)					
			s_{wb}	%[d]	s_{bb}	%	u_{bb}	%
Acetamiprid	0.096 ± 0.004	3.8	0.0024	2.5	0.0029	3.0	0.0007	0.8
Azoxystrobin	0.112 ± 0.003	3.1	0.0026	2.3	0.0024	2.1	0.0008	0.7
Boscalid	0.110 ± 0.005	4.3	0.0031	2.8	0.0037	3.4	0.0009	0.8
Buprofezin	0.098 ± 0.005	4.7	0.0034	3.4	0.0033	3.4	0.0010	1.0
Carbendazim	0.092 ± 0.002	2.2	0.0020	2.1	0.0004	0.4	0.0006	0.6
Difenoconazole	0.109 ± 0.006	5.3	0.0051	4.7	0.0028	2.6	0.0015	1.4
Ethofenprox	0.105 ± 0.006	6.1	0.0042	4.0	0.0049	4.7	0.0013	1.2
Imidacloprid	0.110 ± 0.004	4.1	0.0025	3.5	0.0025	2.3	0.0011	1.0
Pyraclostrobin	0.110 ± 0.005	4.3	0.0037	2.8	0.0037	3.4	0.0009	0.8
Tebuconazole	0.113 ± 0.006	5.3	0.0050	3.2	0.0050	4.4	0.0011	1.0

[a]Calculated by averaging 30 samples taken from the top, middle and bottom of each bottle

[b]Standard deviation

[c]Relative standard deviation: (SD/assigned value) × 100

[d]Percentage of assigned value as a relative value

Table 6 Results of analysis under four different storage conditions

Pesticide	Average ± SD[a] (mg/kg), RSD[b] (%)			
	Room temperature	Cold	Freezing	Deep freezer
Acetamiprid	0.101 ± 0.008 (8.4%)	0.101 ± 0.004 (3.9%)	0.099 ± 0.002 (2.2%)	0.100 ± 0.002 (2.1%)
Azoxystrobin	0.101 ± 0.002 (2.1%)	0.103 ± 0.004 (3.9%)	0.102 ± 0.001 (1.3%)	0.104 ± 0.004 (3.7%)
Boscalid	0.105 ± 0.005 (4.9%)	0.103 ± 0.006 (6.2%)	0.101 ± 0.003 (2.7%)	0.102 ± 0.004 (4.3%)
Buprofezin	0.103 ± 0.004 (3.5%)	0.106 ± 0.003 (2.6%)	0.101 ± 0.005 (4.7%)	0.106 ± 0.002 (2.0%)
Carbendazim	0.085 ± 0.002 (2.1%)	0.085 ± 0.002 (2.7%)	0.092 ± 0.005 (5.5%)	0.088 ± 0.003 (3.8%)
Difenoconazole	0.100 ± 0.006 (6.2%)	0.101 ± 0.006 (5.5%)	0.101 ± 0.004 (3.6%)	0.100 ± 0.005 (4.6%)
Ethofenprox	0.106 ± 0.003 (2.7%)	0.106 ± 0.004 (3.6%)	0.105 ± 0.003 (2.5%)	0.105 ± 0.003 (2.5%)
Imidacloprid	0.099 ± 0.003 (2.6%)	0.098 ± 0.005 (5.0%)	0.097 ± 0.002 (1.9%)	0.103 ± 0.006 (5.7%)
Pyraclostrobin	0.106 ± 0.003 (2.8%)	0.103 ± 0.001 (1.2%)	0.102 ± 0.002 (1.7%)	0.104 ± 0.003 (2.7%)
Tebuconazole	0.105 ± 0.010 (9.3%)	0.114 ± 0.004 (3.9%)	0.109 ± 0.014 (12.8%)	0.115 ± 0.005 (4.0%)

[a]Standard deviation

[b]Relative standard deviation: (SD/average) × 100

Table 7 Stability under room temperature, cold, freezing and deep freezer conditions

Pesticide	Storage conditions[a]	ISO Guide 35			
		b_1	b_0	$s_{(b1)}$	$t_{0.95,\ n-2}\times s_{(b1)}$
Acetamiprid	Room temp.	0.0348	4.8551	0.0061	0.0778
	Cold	0.0055	4.9479	0.0041	0.0522
	Freezing	0.0008	4.9338	0.0029	0.0370
	Deep freezer	0.0005	4.9482	0.0006	0.0074
Azoxystrobin	Room temp.	0.0120	5.0081	0.0087	0.1110
	Cold	0.0020	5.0969	0.0030	0.0381
	Freezing	0.0010	5.0532	0.0003	0.0035
	Deep freezer	0.0016	5.0337	0.0001	0.0017
Boscalid	Room temp.	0.0209	5.1324	0.0267	0.3388
	Cold	− 0.0048	5.2546	0.0152	0.1928
	Freezing	0.0001	5.0548	0.0026	0.0334
	Deep freezer	0.0003	5.0509	0.0004	0.0047
Buprofezin	Room temp.	− 0.0172	5.2179	0.0101	0.1285
	Cold	− 0.0046	5.3568	0.0057	0.0725
	Freezing	− 0.0071	5.2895	0.0014	0.0183
	Deep freezer	0.0008	5.2102	0.0005	0.0062
Carbendazim	Room temp.	− 0.0031	4.2882	0.0001	0.0016
	Cold	0.0031	4.2178	0.0043	0.0549
	Freezing	0.0078	4.3063	0.0011	0.0137
	Deep freezer	− 0.0002	4.4161	0.0011	0.0146
Difenoconazole	Room temp.	0.0204	4.8914	0.0047	0.0593
	Cold	0.0126	4.8262	0.0027	0.0342
	Freezing	0.0029	4.9201	0.0029	0.0369
	Deep freezer	0.0004	4.9717	0.0009	0.0116
Ethofenprox	Room temp.	− 0.0065	5.3365	0.0167	0.2127
	Cold	0.0072	5.1604	0.0061	0.0777
	Freezing	− 0.0012	5.3050	0.0026	0.0336
	Deep freezer	− 0.0001	5.2583	0.0000	0.0001
Imidacloprid	Room temp.	0.0189	4.8531	0.0075	0.0958
	Cold	0.0076	4.7906	0.0017	0.0218
	Freezing	− 0.0001	4.8578	0.0023	0.0290
	Deep freezer	0.0024	4.9224	0.0009	0.0118
Pyraclostrobin	Room temp.	0.0179	5.1983	0.0149	0.1894
	Cold	0.0016	5.1332	0.0002	0.0290
	Freezing	0.0003	5.0865	0.0025	0.0315
	Deep freezer	0.0011	5.1037	0.0002	0.0029
Tebuconazole	Room temp.	0.0046	5.2272	0.0764	0.9706
	Cold	0.0109	5.5220	0.0036	0.0455
	Freezing	− 0.0122	5.8952	0.0.182	0.2314
	Deep freezer	− 0.0004	5.78461	0.00183	0.0232

[a]Storage periods for room temperature, cold, freezing and deep freezer conditions were 12, 40, 70 and 234 days, respectively. The value of '$t_{0.95,\ n-2}$' was 12.71

In conclusion, cabbage reference material containing 10 different pesticides was developed by the Korea Institute of Toxicology for PT and verification of analytical methods used for assessment of multi-residue pesticides. The established analytical procedure was successfully validated by LC–MS/MS, and homogeneity and short- and long-term stability were evaluated at four different temperatures. Homogeneity between and within bottles was within acceptable limits for all 10 pesticides, and no significant decrease occurred during storage for 234 days at − 80 °C.

Our findings could be applied widely to the production of reference material for pesticide analysis and used as a tool to assess the analytical reliability of laboratories.

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References

1. Rotenberg M, Shefi M, Dany S, Dore I, Tirosh M, Almog S (1995) Differentiation between organophosphate and carbamate poisoning. Clin Chim Acta 234:11–21
2. Carvalho FP (2006) Agriculture, pesticides, food security and food safety. Environ Sci Policy 9:685–692
3. Yarita T, Otake T, Aoyagi Y, Kuroda Y, Numata M, Iwata H, Watai M, Mitsuda H, Fujikawa T, Ota H (2014) Development of soybean certified reference material for pesticide residue analysis. Talanta 119:255–261
4. Rawn DF, Cao XL, Doucet J, Davies DJ, Sun WF, Dabeka RW, Newsome WH (2004) Canadian total diet study in 1998; pesticide levels in foods from Whitehorse, Yukon, Canada, and corresponding dietary intake estimates. Food Addit Contam 21:232–250
5. Senyuva HZ, Gilbert J (2006) Assessment of the performance of pesticide-testing laboratories world-wide through proficiency testing. Trends Analyt Chem 25:554–562
6. Grimalt S, Harbeck S, Shegunova P, Seghers B, Sejerøe-Olsen H, Emteborg H, Dabrio M (2015) Development of a new cucumber reference material for pesticide residue analysis: feasibility study for material processing, homogeneity and stability assessment. Anal Bioanal Chem 407:3083–3091
7. COMAR (2016) International database for certified reference materials. http://www.comar.bam.de/en/. Accessed 4 May 2016
8. Otake T, Itoh N, Aoyagi Y, Matsuo M, Hanari N, Otsuka S, Yarita T (2009) Development of certified reference material for quantification of two pesticides in brown rice. J Agric Food Chem 57:8208–8212
9. Otake T, Yarita T, Aoyagi Y, Kuroda Y, Numata M, Iwata H, Watai M, Mitsuda H, Fujikawa T, Ota H (2013) Development of apple certified reference material for quantification of organophosphorus and pyrethroid pesticides. Food Chem 138:1243–1249
10. Medina-Pastor P, Rodriguez-Torreblanca C, Andersson A, Fernandez-Alba AR (2010) European Commission proficiency tests for pesticide residues in fruits and vegetables. Trends Anal Chem 29:70–83
11. Kim Y, Song K, Shin S, Lee J, Jeong G, Hong E, Park J, Yu S (2010) Preparation of wastewater-based reference materials for heavy metal analysis and interlaboratory study. Anal Sci Technol 23:295–303
12. Jung J, Park J, Yoo S, Kweon S, Hong S, Sun Y, Park C, Choi C (2012) A study on the development of phthalate plasticizers CRM in ABS resin. Anal Sci Technol 25:273–283
13. Ahn S, Kim B, Hwang E (2011) Stability monitoring of pesticide residues in a chinese cabbage certified reference material. Bull Korean Chem Soc 32:1365–1367
14. Kim J, Choi S, Oh Y, Kwon Y, Hong S, Sung M, Lee S, Seo J (2016) Development of analytical reference material for proficiency test of pesticide multi-residue analysis in green-pepper. Korean J Pestic. Sci 20:211–220
15. Kim J, Oh Y, Choi S, Hong S, Kim S, Woo I, Kim J, Seo J (2016) Development of analytical reference material for proficiency test of pesticide multi-residue analysis in tomato. Korean J Environ Agric 35:223–233
16. ISO Guide 35 (2006) Reference materials–general and statistical principles for certification. International Organization for Standardization, Geneva
17. ISO 13528 (2005) Statistical methods for use in proficiency testing by interlaboratory comparison. International Organization for Standardization, Geneva
18. Thompson M, Ellison SLR, Wood R (2006) The international harmonized protocol for the proficiency testing of analytical chemistry laboratories. Pure Appl Chem 78:145–196
19. Kim J, Seo J, Moon J, Kim J (2013) Multi-residue method development of 8 benzoylurea insecticides in mandarin and apple using high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. J Korean Soc Appl Biol Chem 56:47–54
20. Linsinger TPJ, Pauwels J, Van der Veen AMH, Schimmel H, Lamberty A (2001) Homogeneity and stability of reference materials. Accred Qual Assur 6:20–25