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Study Analysis of Isocycloseram and Its Metabolites in Agricultural Food Commodities

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Abstract

An accurate and easy-to-use analytical method for determining isocycloseram and its metabolites (SYN549431 and SYN548569) residue is necessary in various food matrixes. Additionally, this method should satisfy domestic and international guidelines (Ministry of Food and Drug Safety and Codex Alimentarius Commission CAC/GL 40). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was used to determine the isocycloseram and its metabolites residue in foods. To determine the residue and its metabolites, a sample was extracted with 20 mL of 0.1% formic acid in acetonitrile, 4 g magnesium sulfate anhydrous and 1 g sodium chloride and centrifuged (4,700 G, 10 min, 4°C). To remove the interferences and moisture, d-SPE cartridge was performed before LC-MS/MS analysis with C_{18} column. To verify the method, a total of five agricultural commodities (hulled rice, potato, soybean, mandarin, and red pepper) were used as a representative group. The matrix-matched calibration curves were confirmed with coefficients of determination (R^2) ≥ 0.99 at a calibration range of 0.001-0.05 mg/kg. The limits of detection and quantification were 0.003 and 0.01 mg/kg, respectively. Mean average recoveries were 71.5-109.8% and precision was less than 10% for all five samples. In addition, inter-laboratory validation testing revealed that average recovery was 75.4-107.0% and the coefficient of variation (CV) was below 19.4%. The method is suitable for MFDS, CODEX, and EU guideline for residue analysis. Thus, this method can be useful for determining the residue in various food matrixes in routine analysis.

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Introduction

Effective pest control has been a challenge in throughout the history of agricultural cultivation. Recent regulatory requirements and public consideration demand that mode of action of pesticides should be favourable for environment and user and more efficiency with more selectivity (Umetsu & Shirai, 2020). That is the reason many newly developed pesticides target molecular sites of pests, not one of plants or weeds such as Acetylcholine Receptor, Ryanodine Receptor, Sodium channel, and GABA channel (Rohrer & Arena, 1995). Approximately, 80% of globally sold insecticides are based on this mechanisms (Jeschke, 2021).

Isocycloseram ($C_{23}H_{18}O_4N_3Cl_2F_4$, IUPAC name: 4-[(5 RS)-5-(3,5-dichloro-4-fluorophenyl)-4,5-dihydro-5-(trifluoromethyl)isoxazol-3-yl]-N-[(4RS)-2-ethyl-3-oxoisoxazolidin-4-yl]-o-toluamide) is a newly developed isoxazoline insecticide and acaricide targeting against the gamma-aminobyturic acid (GABA)-gated chloride channel from Syngenta Crop Protection (Basel, Switzerland). Isoxazolines structure are five-membered heterocyclic compounds containing N (Nitrogen) and O (Oxygen) atoms (Luo et al., 2022). GABA is an import inhibitory neurotransmitter in invertebrates (Cassayre, 2021). Thus, blocking of the GABA-gated channel promotes reducing neutral inhibition and results in hyper-excitation of the central nervous system, convulsions and eventually death (Bloomquist, 2003; Delgado et al., 2019). The first developed chemicals of this group were Cyclodiend and Phenylpyrazole (Jeschke, 2021; Ozoe et al., 2013). However, these chemicals exhibited receptor mutation and co-resistance with other pesticides. Mutations of M2 membrane-spanning region (A2'S and A2'G) result in reduced chemical efficiency and cross-resistance to other channel antagonists such as fipronil and ethiprole (Ozoe et al., 2013). Currently, they belong to Insecticide Resistance Action Committee (IRAC) group 2A (http://irac-online.org/modes-of-action). To overcome these disadvantages, new mode of action related to GABA targeting was developed. That is GABA-gated chloride channel allosteric modulators which act as noncompetitive GABA-gated channel antagonist. Thus, isocycloseram are classified as IRAC 30 named GABA-gated chloride channel allosteric modulator.

According to report data provided by Australian Pesticides and Veterinary Medicines Authority (2022), metabolism studies for SYN549431 ($C_{18}H_{12}O_2N_2Cl_2F_4$, IUPAC name: 4-(5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-2-methylbenzamide) and SYN548569 ($C_8H_4O_2Cl_2F_4$, IUPAC name: 1-(3,5-dichloro-4-fluorophenyl)-2,2,2-trifluoroethanone) were conducted on primary crops (tomato, mustard greens, rice and soybeans). Two metabolite is the residue level was generally <10% when compared to of parent isocycloseram. However, an MRL and residue definition is not established in any countries and we considered to a variety agricultural samples pesticide residues for safety control to future.

Since December 2016 (Ministry of Food and Drug Safety, MFDS notification No. 2015-78('15.10.29)), Korea has been enforced Positive List System for "nuts/seeds and tropical fruits" and expend to all agricultural commodities on 2019 (MFDS notification No. 2018-8('18.02.22)). Only food commodities should be handling with legally registered pesticide to cross the border of Korea. Furthermore, the numbers of inspection for imported foods are increased every year to insecure food safety. Based on MFDS data (2022), imported food are increased more than 20% only 3-year flame (from 2017 to 2020). As the results, the number of inspections should be enlarged because MFDS use certain percentage for food inspection. It means that developing routinely use analytical method with simple and easy steps is necessary to cover several agricultural commodities.

The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) has been recognized as an efficient analysis method for multiclass pesticide residue since developed in 2013 (Anastassiades et al., 2003; Islam et al., 2021). Before applying analysis machines such as gas chromatography (GC) and liquid chromatography (LC), two components (extraction and cleanup) are the main step of QuEChERS. LC-MS/MS is a main instrument for detection of pesticide residue with high sensitivity and selectivity. Additionally, this method is heavily used for routine multidrug detection because it can effectively detect acidic pesticides and low volatile pesticides, which gas chromatograph cannot work easily (Assalin et al., 2014; Pareja, Cesio et al., 2011; Pareja, Martínez-Bueno et al., 2011; Zheng et al., 2018).

Several studies have been reported about modifications of original QuEChERS for better analysis of sample matrix and chemicals (Islam et al., 2018; Islam et

al., 2019; Islam et al., 2021; Zheng et al., 2018). Even, Association of Official Agricultural Chemists (AOAC, 2007) and the Committees of European Normalization methods are available as other types of modified QuEChERS.

The purpose of this study is investigating analytical method determining isocycloseram and its metabolites (SYN549431 and SYN548569) residue in agricultural matrixes. This method should be meet domestic and international guideline (Ministry of Food and Drug Safety, Codex Alimentarius Commission CAC/GL 40 and EU SANTE/12682/2019) for utilizing routinely and east-to-use methods for monitoring food safety.

Materials and Methods

Reagents and Materials

Isocycloseram (purity 98.0%) and its metabolites SYN 549431 (98.0%) and SYN548569 (98.6%) were supplied by Syngenta Crop Protection (Basel, Switzerland) and their chemical structures and characters are shown in Fig. 1 and Table 1. HPLC grade of acetonitrile and formic acid and LC/MC grade of water were purchased from Merck (Darmstadt, Germany). For extraction and purification, three extraction kits (Original, No. 5982-0500; EN, No. 5982-5650; AOAC, No. 5982-5755) and five purification kits (No. 5982-4921, containing 25 mg primary secondary amine (PSA)/150 mg $MgSO_4$; No. 5982-5021, containing 150 mg $MgSO_4$/25 mg octadecysilyl (C_{18}); No. 5982-5121, containing 25 mg PSA/150 mg $MgSO_4$/25 mg C_{18}; No. 5982-5221, containing 25 mg PSA/150 mg $MgSO_4$/2.5 mg graphitized carbon black (GCB); No. 5982-5421, containing 50 mg PSA/ 150 mg $MgSO_4$/50 mg GCB/50 mg C_{18}) were purchased by Agilent Technologies (Santa Clara, CA, USA). Other reagents used in this study were purchased from Wako

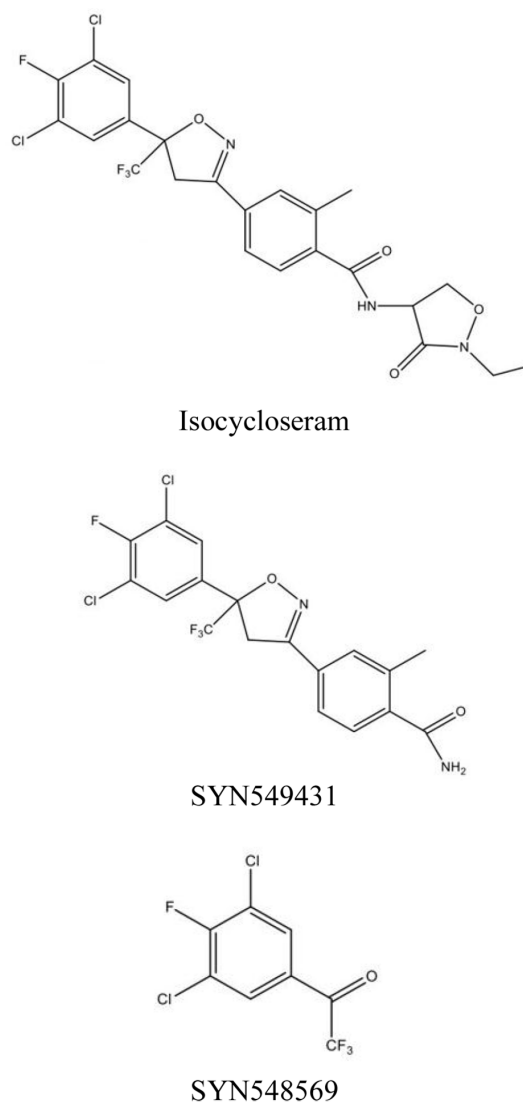Isocycloseram

SYN549431

SYN548569

Fig. 1. Molecular structures of isocycloseram and its metabolites.

Pure chemical industries (Osaka, Japan), Merck (Darmstadt, Germany), or Sigma Aldrich (St. Louis, MO, USA). All reagents and organic solvents were of analytical

Table 1. Physicochemical characteristics of isocycloseram and its metabolites

Property	Isocycloseram	SYN549431	SYN548569
CAS no.	2061933-85-3	-	1190865-44-1
Molecular formula	$C_{23}H_{19}Cl_2F_4N_3O_4$	$C_{18}H_{12}Cl_2F_4N_2O_2$	$C_8H_2Cl_2F_4O$
Molecular weight (g/mL)	548.3	435.2	261.0
Exact mass (Da)	547.1	434.0	260.0
Density (g/cm³)	-	-	1.6 ± 0.1
Boiling point (°C)	-	-	282.5 ± 40.0[a]
Melting point (°C)	-	-	N/A
LogP	-	-	4.13

[a] at 760 mmHg.

grade. Organic (non-pesticide treated) samples of these five representative (hulled rice, potato, soybean, mandarin and red pepper) agricultural commodities were purchased from local markets (Seoul, Korea). Briefly, the samples (at least 1 kg per commodity) were chopped, homogenized, and kept at -20°C until further analysis.

Standard Solution

Each stock solution (approximately 1,000 mg/L) was prepared in acetonitrile. Briefly, 20.23 mg isocycloseram, 20.40 mg SYN549431, and 20.28 mg SYN 548569 were dissolved into each 20 mL of acetonitrile. The intermediate working solution (0.25 μg/mL) were prepared by dilution in acetonitrile. The matrix-matched solutions were prepared that concentration levels (0.001, 0.0025, 0.01, 0.025, and 0.05 μg/mL) by dilution include more than 90% of the matrix. All solutions were stored in amber vials at 4°C until further analysis.

pH Condition of Extraction

Extraction efficiency was compared depending on pH conditions. Four different pH conditions were made by 0.1%, 1%, 2%, and 5% formic acid in acetonitrile for QuEChERS extraction procedure.

LC−MS/MS Conditions

LC-MS/MS system, consisted of Acuity UPLC (Waters, Milford, MA) and Xevo TQ-S tandem quadruple mass spectrometer (Waters, Milford, MA), were used in this study. LC separation was carried out in Cadenza CD-C$_{18}$ HT column (2.0 mm I.D. × 150 mm L., 3.0 μm) at a temperature of 40°C. The injection volume and flow rate were 5.0 μL and 0.4 mL/min, respectively. MS/ MS was operated using ESI (electrospray ionization) positive mode and negative

mode. The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B). The initial mobile phase of 10/90 (A/B, v/v) was retained for 0.5 min, changed to 95/5 (A/B, v/v) over 4 min and then held for 4 min, and finally changed to 10/90 (A/B, v/v) over 0.1 min and then retained until 12 min. Analytical condition and selected-ion for detection of these chemical were described more detail in Table 2.

Method Validation

A validation of this new method was investigated to confirm its reliability. The tests looked for several facts such as accuracy, precision, limits of quantization (LOQ) and linearity. To validate accuracy and precision, recovery test with spiking chemicals samples was investigated with range of 0.001-0.05 mg/kg.

Matrix-matched calibration curves were prepared by combination of standard solution and matrix extracts. After confirming no interference from non-pesticide treated samples, limits of quantization (LOQ) were calculated based on limits of detection quantization (LOD) and concentration ratio. Method validation was assessed following the EU SANTE/12682/2019 (European Commission, 2019) and MFDS guideline, according to which reliability, precision and recovery should be in the range 70-120% with relative standard deviation ≤ 20%.

Results and Discussion

Evaluation of the QuEChERS Method

Recently, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method was heavily used because it has several advantages such as reduction of solvent usage, time and effort (Anastassiades et al., 2003; Islam et al., 2018; Schenck & Hobbs, 2004). This method

Table 2. Selected ion for the determination of isocycloseram and its metabolites

Compound	Retention time (min)	Molecular weight	Exact mass	Precursor ion (m/z)	Product ion (m/z)	CE[a] (eV)
Isocycloseram	4.7	548.3	547.1	548.1	418.0[b]	21
					160.0	43
SYN549431	4.5	435.2	434.0	435.1	160.1[b]	37
					392.0	23
SYN548569	4.0	261.0	260.0	261.0	191.0[b]	-14
				263.0	193.0	-14

[a]Collision energy(eV), [b]Quantification ion.

shows great analysis for samples with different characteristics (Islam et al., 2021). Basically, QuEChERS method increases the efficiency of extraction by separation from unwanted interference and water with usages of acetonitrile and addition of anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) based on "salting-out" principle (Lehotay, 2007). Additionally, Association of Official Agricultural Chemical International (AOAC) method developed from AOAC used $MgSO_4$ and sodium acetate, while EN method developed from European Committee for Standardization used citric acid (Lehotay et al., 2005; Roussev et al., 2019). In this study, we investigate three methods, original QuEChERS and two modified ones (AOAC and EN) to compare the recovery rates using different salts. Meanwhile, we tested hulled rice only for set-up sample preparation steps, because it contained more oil and organic acids compared to other representative agricultural commodities. Overall recovery from original QuEChERS methods provided the best result than other two methods in hulled rice (Table 3). For isocycloseram, recovery percentage showed 100.8% while 82.4% and 95.5% from AOAC and EN. For SYN549431, AOAC (86.0%) showed better results than EN (84.9%), still lower than original method (93.2%). In the case of SYN548569, original method (108.1%) showed significantly better recovery than other two methods (72.4% and 77.3%). In sum, original QuEChERS method was chosen for further investigation for isocycloseram and two metabolites in this study.

Investigation of the Optimal Extraction pH

Extraction is also the key step in QuEChERS method development. Extraction is influenced by many parameters such as pH, sample volume and extraction time (Musa et al., 2018). In this study, pH conditions for extraction were tested. Extraction of isocycloseram and its metabolites were investigated in four different pH conditions (0.1%, 1%, 2%, and 5% formic acid in acetonitrile, Table 4). Generally, higher pH helped all three chemical extractions. Acetonitrile is most commonly used extraction solvent because this has been used for LC-MS/MS detection. Average pH of solvent was 8.0 (pure acetonitrile), 3.0 (1% formic acid), and 2.5 (5% formic acid) (Islam et al., 2021). In sum, recovery might be optimal between pH 8.0 and pH 3.0.

Selection of the Optimal d-SPE Sorbent for Sample Cleanup

Usage of proper sorbent leads to increase detection performance with sufficient clean-up. Dispersive solid phase extraction (d-SPE) is commonly used purification method. Compared to cartridge-mediated method, it shows high efficiency in the view of time, effort, and cost. To increase the purification efficiency, several combinations of absorbents were investigated: $MgSO_4$, PSA, C_{18}, and GCB. $MgSO_4$ takes moisture from samples, while PSA removes organic acids, fatty acid, and carbohydrate (Shida et al., 2015). C_{18} is good for eliminate lipid and non-polar interference and GCB for pigments and sterols (Shida et al., 2015). Thus, depending

Table 3. The recovery results of isocycloseram and its metabolites according to three QuEChERS method in hulled rice

Compound	Recovery ± RSD[a] (%)		
	Original	AOAC	EN
Isocycloseram	100.8 ± 0.9	82.4 ± 0.1	95.5 ± 0.9
SYN549431	93.2 ± 0.6	86.0 ± 4.1	84.9 ± 0.5
SYN548569	108.1 ± 6.4	72.4 ± 17.9	77.3 ± 1.4

[a]Relative standard deviation from three repeated measurements.

Table 4. The extraction efficiency of isocycloseram and its metabolites according to pH of extraction solvent using QuEChERS method in hulled rice

Compound	Extraction solvent			
	Recovery ± RSD[a] (%)			
	0.1% F.A[b] in acetonitrile	1% F.A in acetonitrile	2% F.A in acetonitrile	5% F.A in acetonitrile
Isocycloseram	100.8 ± 0.9	89.3 ± 3.2	91.9 ± 4.4	74.9 ± 1.9
SYN549431	93.2 ± 0.6	85.2 ± 2.5	80.7 ± 13.0	80.3 ± 7.4
SYN548569	108.1 ± 6.4	86.9 ± 4.2	88.6 ± 32.4	77.1 ± 21.9

[a]Relative standard deviation from three repeated measurements, [b]Formic Acid.

on matrix characteristic, choice of absorbents helps to optimize the clean-up process.

In this study, five different combinations of sorbents (25 mg PSA/150 mg MgSO₄, 25 mg PSA/150 mg MgSO₄/25 mg C₁₈, 25 mg PSA/150 mg MgSO₄/2.5 mg GCB, 50 mg PSA/150 mg MgSO₄/50 mg GCB/50 mg C₁₈, 25 mg C₁₈/150 mg MgSO₄) were tested for purification efficiency. Combination of 25 mg PSA/150 mg MgSO₄ and 25 mg C₁₈/150 mg MgSO₄ showed the better adsorption efficiency than other three combinations (Table 5). Both PSA and C₁₈ are good for removing oily component form samples. Thus, these sorbents might provide better recovery. Sorbent combination of 25 mg PSA/150 mg MgSO₄/25 mg C₁₈ provided lower purification of SYN548569, while the mixture of sorbent 25 mg PSA/150 mg MgSO₄/2.5 mg GCB caused lower efficiency for SYN548569. And, 50 mg PSA/150 mg MgSO₄/50 mg GCB/50 mg C₁₈ showed less than 50% purification of isocycloseram and SYN549431.

With hulled rice matrix, recovery results of isocycloseram and its metabolites were not enough to reach analysis guideline (70-120%) by using 25 mg PSA/150 mg MgSO₄. When repeated 3 times at 3 concentrations (Table 6). This combination showed low recovery rate, even non-detectable for isocycloseram, with the lowest spiking amount (0.01 mg/kg) of chemicals: 61.6% and 69.0% for SYN549431 and SYN548569. As the result,

sorbent combination of 25 mg C₁₈/150 mg MgSO₄ was used for further analysis. Both PSA and C₁₈ are good for removing oily component form samples. These sorbents could be the good candidates for hulled rice samples. However, PSA showed less clean-up outcome than C₁₈ with hulled rice sample.

Method Validation

To exam the analytical performance of developed method, limited of detections (LOD) quantification (LOQ), linearity, selectivity, recovery (accuracy and precision) was investigated. First, LOD and LOQ were determined by the ratio of signal-to-noise (S/N). LOD was defined as three times higher than the level of noise (0.0075 μg/mL, S/N ≥ 3), and LOQ were calculated as the concentrations ten times of the noise (0.0025 μg/mL, S/N ≥ 10). When we consider dilution factors (4 = 20 mL/5 g), the value of LOD and LOQ were 0.003 and 0.0025 mg/kg, respectively.

Matrix components could be consideration point for developing detection method because they can interference analyte signal. Thus, matrix-matched calibration has been used for confirming whether matrix component alter signal (Kirchner et al., 2008; Surma et al., 2014) matrix-matched calibration curve were constructed by plotting the peak area vs. the five concentrations (0.001, 0.0025, 0.01, 0.025, and 0.05 mg/kg) of the iso

Table 5. Recovery with different condition of sorbent for isocycloseram and its metabolites adsorption in hulled rice and mandarin

d-SPE	Recovery ± RSD[a] (%)		
	Compound	Hulled rice	Mandarin
25 mg PSA + 150 mg MgSO₄	Isocycloseram	102.4 ± 3.2	104.1 ± 0.8
	SYN549431	89.1 ± 2.7	98.9 ± 0.9
	SYN548569	76.3 ± 4.3	85.9 ± 5.2
25 mg PSA + 150 mg MgSO₄ + 25 mg C₁₈	Isocycloseram	103.3 ± 5.7	93.8 ± 16.7
	SYN549431	90.7 ± 4.8	92.6 ± 12.0
	SYN548569	66.2 ± 9.3	57.6 ± 9.0
25 mg PSA + 150 mg MgSO₄ + 2.5 mg GCB	Isocycloseram	93.4 ± 3.4	89.9 ± 0.8
	SYN549431	95.8 ± 0.7	87.9 ± 1.0
	SYN548569	98.3 ± 7.2	52.5 ± 27.8
50 mg PSA + 150 mg MgSO₄ + 50 mg GCB + 50 mg C₁₈	Isocycloseram	30.0 ± 4.1	6.8 ± 4.5
	SYN549431	39.4 ± 1.5	14.9 ± 2.2
	SYN548569	61.8 ± 3.3	208.3 ± 11.8
150 mg MgSO₄ + 25 mg C₁₈	Isocycloseram	89.5 ± 2.7	96.2 ± 4.1
	SYN549431	86.1 ± 3.2	92.6 ± 6.3
	SYN548569	80.0 ± 7.6	78.2 ± 24.2

[a]Relative standard deviation from three repeated measurements.

Table 6. The recovery test of isocycloseram and its metabolites in hulled rice

Matrix	Compound	Purification kit : PSA + MgSO$_4$	
		Spiking (mg/kg)	Recovery ± RSD[a] (%)
Hulled rice	Isocycloseram	0.01	69.0 ± 5.5
		0.1	99.0 ± 2.7
		0.5	92.2 ± 3.4
	SYN549431	0.01	61.6 ± 7.1
		0.1	90.1 ± 1.4
		0.5	85.6 ± 2.6
	SYN548569	0.01	N.D.[b]
		0.1	71.5 ± 17.0
		0.5	73.9 ± 2.4

[a]Relative standard deviation from three repeated measurements, [b]Not determined.

cycloseram and its metabolites. Good linearity for iso-cycloseram and metabolites ($R^2 \geq 0.99$) was observed for all five food matrices.

The selectivity was confirmed by comparing representative chromatogram of standard working solution, blank, and fortified sample extracts. No endogenous components were observed at the retention time and m/z of the analyte. As the result, this method is confirmed to have a good separation and selectivity (Fig. 2). Also, recovery test was performed with three different levels (1, 10, and 50-fold of LOQ), 0.01, 0.1 and

0.5 mg/kg for testing accuracy and precision. Each test was done with five replicates. Recovery was obtained 71.5-109.8% between with RSD 0.9-10.8%. Finally, inter-laboratory validation results were testing Seoul regional Food and Drug Administration to confirm the Validity of the developed method (Table 7-9). These results show an average recovery of 75.4-107.0% and coefficient of variation (CV) was below 19.4% and guided by EU SANTE/12682/2019 (European Commission, 2019), CODEX and MFDS, which means recovery should fall between 70-120% with RSD $\leq$ 20%.
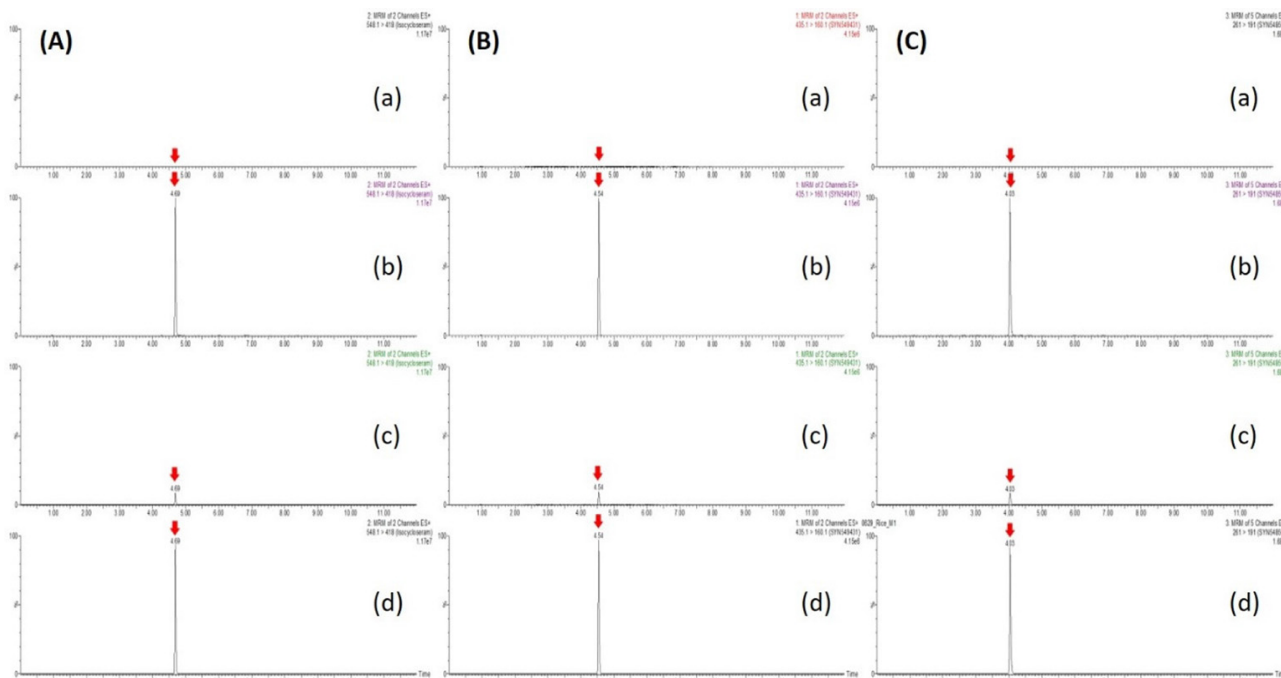

Fig 2. Representative MRM LC-MS/MS chromatograms of (A) Isocycloseram, (B) SYN549431 and (C) SYN548569 at (a) blank sample, (b) matrix-matched standard (0.025 mg/L) (c) recovery sample spiked (0.01 mg/kg), and (d) recovery sample spiked (0.1 mg/kg) in hulled rice.

Table 7. Validation results for the determination isocycloseram in samples

Compound	Fortification (mg/kg)	Recovery ± RSD[a] (%)		Ave.[b] (%)	CV[c] (%)
		Intra-Day	Inter-Day		
Hulled rice	0.01	95.6 ± 9.5	87.4 ± 10.3	92.5	10.2
	0.1	94.5 ± 2.1	75.4 ± 9.6	87.3	12.3
	0.5	78.1 ± 1.6	76.8 ± 10.1	77.6	5.5
Potato	0.01	109.8 ± 2.4	103.8 ± 2.5	107.6	3.7
	0.1	103.0 ± 2.9	91.9 ± 0.8	98.9	6.3
	0.5	92.7 ± 2.0	92.5 ± 5.4	92.6	3.3
Soybean	0.01	104.7 ± 2.3	96.7 ± 3.5	101.7	4.8
	0.1	105.3 ± 2.8	89.0 ± 5.3	99.2	9.2
	0.5	92.8 ± 4.0	86.2 ± 0.7	90.3	4.9
Mandarin	0.01	84.1 ± 9.0	90.6 ± 4.9	86.5	8.1
	0.1	75.6 ± 4.5	92.1 ± 4.8	81.8	11.2
	0.5	74.6 ± 3.9	90.6 ± 1.8	80.6	10.7
Red pepper	0.01	105.4 ± 3.0	87.1 ± 5.6	98.5	10.3
	0.1	107.3 ± 5.2	88.5 ± 5.8	100.3	10.9
	0.5	97.4 ± 5.7	89.4 ± 6.4	94.4	7.0

[a]Recovery values for 5 (Lab1) or 3 (Lab2) repetitions with relative standard deviation, [b]Average recovery of inter-laboratory, [c]Average coefficient of variation of inter-laboratory.

Table 8. Validation results for the determination SYN549431 in samples

Compound	Fortification (mg/kg)	Recovery ± RSD[a] (%)		Ave.[b] (%)	CV[c] (%)
		Intra-Day	Inter-Day		
Hulled rice	0.01	88.1 ± 6.4	84.1 ± 2.6	86.6	5.6
	0.1	86.9 ± 2.0	94.8 ± 3.6	89.9	5.2
	0.5	71.5 ± 1.7	92.6 ± 19.4	79.4	18.4
Potato	0.01	109.6 ± 3.7	99.3 ± 7.7	105.7	7.0
	0.1	102.0 ± 3.6	100.6 ± 6.7	101.5	4.5
	0.5	91.1 ± 3.1	96.9 ± 15.5	93.3	9.4
Soybean	0.01	97.0 ± 3.5	87.0 ± 4.1	93.3	6.5
	0.1	98.8 ± 0.9	98.5 ± 10.3	98.7	5.6
	0.5	89.2 ± 9.2	84.1 ± 3.9	91.4	7.9
Mandarin	0.01	87.8 ± 8.4	101.5 ± 6.1	92.9	10.3
	0.1	83.5 ± 5.4	100.7 ± 3.3	90.0	10.8
	0.5	79.6 ± 4.1	99.1 ± 6.6	86.9	12.6
Red pepper	0.01	103.1 ± 3.0	106.1 ± 3.3	104.3	3.3
	0.1	102.3 ± 4.0	88.4 ± 7.9	97.1	8.9
	0.5	96.1 ± 7.7	85.7 ± 8.4	92.2	9.4

[a]Recovery values for 5 (Lab1) or 3 (Lab2) repetitions with relative standard deviation, [b]Average recovery of inter-laboratory, [c]Average coefficient of variation of inter-laboratory.

Conculsions

In this study, a modification for the QuEChERS method of isocycloseram in crops was developed and validated. For extraction, 0.1% formic acid in acetonitrile was used for optimal pH, and the combination of 4 g $MgSO_4$ and 1 g NaCl were added as sorbents. For purification, 150 mg $MgSO_4$ and 25 mg C_{18} were used in

Table 9. Validation results for the determination SYN548569 in samples

Compound	Fortification (mg/kg)	Recovery ± RSD[a] (%)		Ave.[b] (%)	CV[c] (%)
		Intra-Day	Inter-Day		
Hulled rice	0.01	106.9 ± 10.8	78.3 ± 3.0	96.2	17.9
	0.1	105.5 ± 3.3	88.9 ± 4.0	99.3	9.2
	0.5	104.0 ± 1.5	96.2 ± 2.1	101.1	4.3
Potato	0.01	93.9 ± 4.3	86.4 ± 9.9	91.1	7.4
	0.1	83.5 ± 5.4	92.2 ± 9.9	86.7	8.6
	0.5	93.3 ± 5.6	96.4 ± 10.7	94.4	7.4
Soybean	0.01	102.3 ± 3.6	78.6 ± 9.5	93.4	14.1
	0.1	93.3 ± 3.5	81.4 ± 18.7	88.8	11.8
	0.5	89.6 ± 4.0	82.1 ± 0.8	86.8	5.5
Mandarin	0.01	97.6 ± 3.2	99.9 ± 7.7	98.5	5.0
	0.1	85.3 ± 2.6	107.0 ± 7.0	93.5	12.9
	0.5	90.9 ± 4.7	97.9 ± 2.8	93.5	5.4
Red pepper	0.01	76.8 ± 9.6	96.2 ± 8.6	84.1	14.7
	0.1	82.0 ± 6.2	98.1 ± 2.2	88.0	10.5
	0.5	86.1 ± 7.8	94.0 ± 1.0	89.1	7.3

[a]Recovery values for 5 (Lab1) or 3 (Lab2) repetitions with relative standard deviation, [b]Average recovery of inter-laboratory, [c]Average coefficient of variation of inter-laboratory.

d-SPE. The extract was successfully analyzed with LC-MS/MS. The validations of this newly developed method were done with recovery ranging from 71.5% to 109.8% with RSD ≤ 20%. The linear correlation coefficient of the calibration curve was ≥ 0.99, and the value of LOD and LOQ were 0.003 and 0.0025 mg/kg, respectively. This method could be suitable for routine analysis for detecting isocycloseram and its metabolites forms in several agricultural commodities.

Note

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Acknowledgements

References

1. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International, 86(2), 412-431. https://doi.org/10.1093/jaoac/86.2.412.

2. Assalin MR, Queiroz SC, Ferracini VL, Oliveira T, Vilhena E, Mattos MLT (2014) A method for determination of imazapic and imazethapyr residues in soil using an ultrasonic assisted extraction and LC-MS/MS. Bulletin of Environmental Contamination and Txicology, 93, 360-364. https://doi.org/10.1007/s00128-014-1330-6.

3. Bloomquist JR (2003) Chloride channels as tools for developing selective insecticides. Archives of Insect Biochemistry and Physiology, 54(4), 145-156. https://doi.org/10.1002/arch.10112.

4. Cassayre J (2021) Crop protection chemistry: Innovation with purpose. Chimia, 75(6), 561-561. https://doi.org/10.2533/chimia.2021.561.

5. Salgado Delgado MA, Usai L, Pan Q, Strømman AH (2019) Comparative life cycle assessment of a novel Al-Ion and a Li-Ion battery for stationary applications. Materials, 12, 3270. https://doi.org/10.3390/ma12233893.

6. Pihlström T, Fernández-Alba AR, Gamón M, Amate CF, Poulsen ME, Lippold R, Anastassiades M (2017) Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. pp. 21-22, Sante, European Commission.

7. Islam AKMM, Hong SM, Lee HS, Moon BC, Kim DB,

Kwon HY (2018) Identification and characterization of matrix components in spinach during QuEChERS sample preparation for pesticide residue analysis by LC-ESI-MS/MS, GC-MS and UPLC-DAD. Journal of Food Science and Technology, 55(10), 3930-3938. https://doi.org/10.1007/s13197-018-3318-4.

8. Islam AKMM, Lee HS, Ro JH, Kim DB, & Kwon HY (2019) Application of high-surface-area graphitized carbon black with primary secondary amine as an alternative quick, easy, cheap, effective, rugged, and safe cleanup material for pesticide multi-residue analysis in spinach. Journal of Separation Science, 42(14), 2379-2389. https://doi.org/10.1002/jssc.201900066.

9. Islam AKMM, Noh HH, Ro JH, Kim DB, Oh MS, Son K, Kwon, HY (2021) Optimization and validation of a method for the determination of acidic pesticides in cabbage and spinach by modifying QuEChERS procedure and liquid chromatography– tandem mass spectrometry. Journal of Chromatography B, 1173, 122667. https://doi.org/10.1016/j.jchromb.2021.122667.

10. Jeschke P (2021) Status and outlook for acaricide and insecticide discovery. Pest Management Science, 77(1), 64-76. https://doi.org/10.1002/ps.6084.

11. Dömötörová M, Matisová E (2008) Fast gas chromatography for pesticide residues analysis. Journal of Chromatography A, 1207(1-2), 1-16. https://doi.org/10.1016/j.chroma.2007.08.089.

12. Lehotay SJ, Maštovská K, Lightfield AR (2005) Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. Journal of AOAC International, 88(2), 615-629. https://doi.org/10.1093/jaoac/88.2.615.

13. Lehotay SJ (2007) Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. Journal of AOAC International, 90(2), 485-520. https://doi.org/10.1093/jaoac/90.2.485.

14. Luo J, Bian C, Rao L, Zhou W, Li Y, Li B (2022) Determination of the residue behavior of isocycloseram in Brassica oleracea and soil using the QuEChERS method coupled with HPLC. Food Chemistry, 367, 130734. https://doi.org/10.1016/j.foodchem.2021.130734.

15. Musa M, Ibrahim WAW, Marsin FM, Keyon ASA, Nodeh HR (2018) Graphene-magnetite as adsorbent for magnetic solid phase extraction of 4-hydroxybenzoic acid and 3, 4-dihydroxybenzoic acid in sting-

less bee honey. Food Chemistry, 265, 165-172. https://doi.org/10.1016/j.foodchem.2018.04.020.

16. Ozoe Y, Kita T, Ozoe F, Nakao T, Sato K, Hirase K (2013) Insecticidal 3-benzamido-N-phenylbenzamides specifically bind with high affinity to a novel allosteric site in housefly GABA receptors. Pesticide Biochemistry and Physiology, 107(3), 285-292. https://doi.org/10.1016/j.pestbp.2013.09.005.

17. Pareja L, Cesio V, Heinzen H, Fernández-Alba AR (2011) Evaluation of various QuEChERS based methods for the analysis of herbicides and other commonly used pesticides in polished rice by LC-MS/MS. Talanta, 83(5), 1613-1622. https://doi.org/10.1016/j.talanta.2010.11.052.

18. Pareja L, Martínez-Bueno MJ, Cesio V, Heinzen H, Fernández-Alba AR (2011) Trace analysis of pesticides in paddy field water by direct injection using liquid chromatography-quadrupole-linear ion trap-mass spectrometry. Journal of Chromatography A, 1218(30), 4790-4798. https://doi.org/10.1016/j.chroma.2011.02.044.

19. Rohrer SP, Arena JP (1995) Ivermectin interactions with invertebrate ion channels, in: Molecular action of insecticides on ion channels. 17, pp. 264-283, Amercian Chemical Society Publications, USA.

20. Roussev M, Lehotay SJ, Pollaehne J (2019) Cryogenic sample processing with liquid nitrogen for effective and efficient monitoring of pesticide residues in foods and feeds. Journal of Agricultural and Food Chemistry, 67(33), 9203-9209. https://doi.org/10.1021/acs.jafc.9b04006.

21. Schenck FJ, Hobbs JE (2004) Evaluation of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach to pesticide residue analysis. Bulletin of Environmental Contamination and Toxicology, 73(1), 24-30. https://doi.org/10.1007/s00128-004-0388-y.

22. Shida SS, Nemoto S, Matsuda R (2015) Simultaneous determination of acidic pesticides in vegetables and fruits by liquid chromatography—Tandem mass spectrometry. Journal of Environmental Science and Health, Part B, 50(3), 151-162. https://doi.org/10.1080/03601234.2015.982381.

23. Surma MK, Sadowska-Rociek AB, Cieślik EJ (2014) Evaluation of the QuEChERS method with GC-MS detection for the determination of organochlorine pesticides in food of animal origin. Food Analytical Methods, 7(2), 366-376. https://doi.org/10.1007/s12161-013-9635-3.

24. Umetsu N, Shirai Y (2020) Development of novel pes-

ticides in the 21st century. Journal of Pesticide Science, 45(2), 54-74. https://doi.org/10.1584/jpestics.D20-201.

25. Zheng W, Park JA, Abd El-Aty AM, Kim SK, Cho SH, Choi JM, Yi H, Cho SM, Ramadan A, et al. (2018) Development and validation of modified QuEChERS method coupled with LC-MS/MS for simultaneous determination of cymiazole, fipronil, coumaphos, fluvalinate, amitraz, and its metabolite in various types of honey and royal jelly. Journal of Chromatography B, 1072, 60-69. https://doi.org/10.1016/j.jchromb.2017.11.011.