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Development and Application of a Multi-Residue Method to Determine Pesticides in Agricultural Water Using QuEChERS Extraction and LC-MS/MS Analysis

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Abstract: Agricultural water is closely linked to surface and ground water as well as soil; hence, ensuring its safety is an important endeavor. We used the "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method to analyze multi-residue pesticides in agricultural water by using a combined-sorbent-based clean-up procedure. Among the various sorbents examined, clean-up using ENVI-Carb combined with a primary secondary amine sorbent delivered the highest recovery of multi-residue pesticides (>93.9%). While the developed method showed satisfactory linearity ($R^2 > 0.9991$), precision, and specificity, recovery was low for pyrazolate (29.1%) and thidiazuron (59.2%). The limits of detection and quantification for the 55 pesticides targeted in this study were in 0.02–3.0 μg L^{-1} and 0.1–9.9 μg L^{-1}, respectively. The developed method was used to identify and quantify multi-residue pesticides during sample analysis. The results suggest that the QuEChERS method employing a combination of ENVI-Carb and another sorbent can be applied for the effective analysis of multi-residue pesticides in agricultural water.

Keywords: agricultural water; clean-up procedure; combined sorbents; ENVI-Carb; QuEChERS

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1. Introduction

Water, an environmental reservoir, has continuously been contaminated by pesticides used for agriculture [1]. Such residual pesticides that remain in agricultural water may have several undesirable effects on soil and crops; these effects tend to vary with water location-ranging from surface and ground water [2,3]. Despite the widespread adverse effects of contaminated water, detection of residual pesticides in agricultural water is difficult because of their low concentrations, wide diversity of chemical classes, and various physicochemical properties and the presence of complex matrices [4]. Therefore, residual pesticides must be strictly controlled to protect the water ecosystem and agricultural produce.

Several analytical methods have been developed for the extraction of residual pesticides from agricultural water; liquid-liquid, solid-phase, and solid-liquid extraction methods are typically used to prepare agricultural water samples [4–6]. However, these methods have disadvantages such as the use of large volumes of organic solvents, inefficient clean-up procedures, and long processing times.

QuEChERS (quick, easy, cheap, effective, rugged, and safe) was developed as an alternative extraction method to overcome these issues [4,5]. Although the QuEChERS method is very rapid and efficient, improvements for reduction of the interference of complex matrices and achievement of satisfactory recovery from sorbents are still desired [7]. In addition, the QuEChERS method is poorly selective and sensitive under the analysis conditions used for some multi-residue pesticides and samples [8].

In the QuEChERS method, the clean-up procedure is considered as an important factor that influences matrix removal and analyte recovery [8,9]. Primary secondary amine (PSA), graphitized carbon black (GCB), zirconium dioxide bonded to silica (Z-Sep), octadecylsilica (C_{18}), and ENVI-Carb are used as sorbents in the QuEChERS method for multi-residue pesticides [10,11]. Among them, GCB is capable of efficiently eliminating steroids and pigments [12,13]; this sorbent was found to be useful for particular class of pesticide, but was inadequate for the clean-up of matrices and the recovery of a diverse number of pesticides [6–9,11–14]. For instance, GCB was known to retain structurally planar pesticides such as chlorothalonil, cyprodinil, fenazaquin, hexachlorobenzene, mepanipyrim, prochloraz, pyrimethanil, quinoxyfen, terbuphos, and thiabendazole [7–10,14]. The use of high amounts of GCB may lead to low recoveries of some planar pesticides [8,13,14]. Nevertheless, the GCB sorbent has been shown to quantitatively recover polar compounds from various environmental water samples [6,8]; however, a method that uses only GCB as the sorbent is insufficiently optimized to effectively remove co-extractives from agricultural water. The use of only GCB as the sorbent leads to low recovery of residual pesticides because of its high affinity for pesticides with planar structures [12,13]. Therefore, previous studies reported that the low-recovery problem can be solved by combining sorbents, including PSA, Z-Sep, and ENVI-Carb, as alternatives to GCB [2,11,15,16].

Herein, we demonstrate that a clean-up method using ENVI-Carb can be used to detect residual pesticides in agricultural water. We developed and validated the method for the multi-residue analysis of 55 pesticides in agricultural water by using liquid chromatography (LC) with mass spectrometry (MS). The results of this study provide an effective strategy for the analysis of residual pesticides in environmental samples such as agricultural water.

2. Materials and Methods

2.1. Chemicals and Reagents

The pesticides listed in Table 1 (>95%) were purchased from AccuStandard (New Haven, CT, USA). A QuEChERS EN extraction kit (P/N 5982-5650), end-capped C_{18} adsorbent (P/N 5982-1382), PSA (P/N 5982-8382), and GCB (AT-5982-4482) were supplied by Agilent Technologies (Santa Clara, CA, USA). Supel™QuE PSA/ENVI-Carb™ (P/N 55176-U), Z-Sep+ (55299-U), ammonium acetate (>99.0%), triphenyl phosphate, $MgSO_4$, and formic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). All solvents were of analytical or high-performance liquid chromatography (HPLC) grade. Each standard stock solutions were prepared to a concentration of 100 μg mL^{-1} using acetonitrile as the solvent. A working standard mixture was prepared from the stock solutions by serial dilution. Stock and working solutions were stored at −20 °C until required for analysis.

Table 1. LC-MS/MS parameter values used during the detection of 55 pesticides.

Pesticides	R.T. (min)	Precursor Ion (*m/z*)	Quantification Transition (*m/z*)	D.P. (V)	C.E. (V)	Confirmatory Transition (*m/z*)	D.P. (V)	C.E. (V)
Amisulbrom	8.9	466.0	227.1	96	27	108.1	96	33
Azinphos-methyl	5.0	318.0	132.0	61	19	160	61	19
Bensulfuron-methyl	5.0	410.9	149.0	66	27	119.1	66	53
Benzobicyclon	5.6	447.0	257.1	91	37	229.1	91	51
Boscalid	5.4	343.0	307.0	101	27	140	106	27
Chlorpyrifos	10.4	350.0	97.0	76	43	198	76	43
Cyclosulfamurom	6.0	422.1	261.1	76	23	218.1	76	37
Diethofencarb	5.2	268.0	226.0	61	15	180	56	23
Diflubenzuron	6.5	311.0	158.1	66	19	141.1	66	45

Table 1. *Cont.*

Pesticides	R.T. (min)	Precursor Ion (*m*/*z*)	Quantification Transition (*m*/*z*)	D.P. (V)	C.E. (V)	Confirmatory Transition (*m*/*z*)	D.P. (V)	C.E. (V)
Diuron	4.8	233.1	72.1	76	35	160.1	76	35
Etoxazole	11.2	360.1	141.0	101	41	304.1	101	27
Etrimfos	7.0	293.1	125.1	76	33	265	76	21
Fenazaquin	11.4	307.2	161.0	111	25	147	111	29
Fenbuconazole	6.4	337.2	125.1	86	41	70	86	35
Fenhexamid	6.1	302.2	55.0	126	59	97.3	91	33
Fenoxaprop-ethyl	9.2	362.1	287.9	96	29	121	96	41
Fenpyroximate	11.3	422.1	366.2	96	25	134.8	96	47
Fludioxonil	5.4	266.0	229.2	36	23	158	36	49
Flufenoxuron	11.2	489.0	158.1	81	27	141.1	81	71
Fluxapyroxad	5.6	382.0	362.0	76	23	341.9	76	31
Forchlorfenuron	4.8	248.1	129.0	71	21	155.1	71	19
Hexaflumuron	8.9	461.0	158.2	81	25	141.1	81	59
Linuron	5.3	249.0	160.0	76	23	182.1	76	19
Lufenuron	10.7	510.9	158.2	59	27	141.2	59	67
Mefenacet	5.8	299.1	148.2	71	19	120.2	71	35
Mepanipyrim	6.0	224.1	76.9	91	61	106	91	39
Metamifop	9.4	441.1	288.0	91	23	180.2	91	27
Methabenzthiazuron	4.7	222.1	165.2	66	23	150.1	66	45
Metrafenone	7.8	409.0	209.1	56	23	227	56	29
Napropamide	6.2	272.2	129.2	76	21	171.2	76	23
Novaluron	9.3	492.7	141.1	76	71	158.1	76	27
Penoxsulam	4.3	484.1	195.0	116	41	163.9	116	49
Pirimiphos-methyl	7.6	306.2	108.2	86	39	164.2	86	29
Probenazole	6.0	224.2	51.1	56	95	63.1	61	103
Propanil	9.1	373.0	302.7	76	23	162.1	76	19
Propaquizafop	5.3	218.1	127.1	76	37	56	71	51
Pyraclostrobin	7.6	388.1	163.0	106	39	193.9	106	19
Pyrazolate	7.9	439.0	91.0	91	71	173	91	29
Pyrazophos	7.8	374.0	222.2	86	27	194.2	86	43
Pyribenzoxim	10.0	610.2	180.0	66	49	413	66	19
Pyriftalid	5.1	319.0	138.9	106	43	83.1	106	67
Pyrimethanil	5.2	200.1	107.0	91	35	82	91	37
Pyrimidifen	9.5	378.1	184.2	86	31	150.2	86	45
Pyriproxyfen	10.2	322.1	96.0	56	23	184.9	56	31
Quinalphos	6.7	299.1	97.1	66	51	163.1	66	33
Quinoclamine	4.0	208.1	105.1	91	33	172	76	29
Quizalofop-ethyl	9.1	373.1	299.2	91	25	163.1	91	59
Sethoxydim	9.6	328.2	178.1	71	25	282.3	71	17
Spinetoram(j)	8.8	748.5	142.2	91	45	98.1	91	101
Spinetoram(L)	9.8	760.5	142.2	96	43	98.2	96	91
Tebufenpyrad	9.7	334.1	144.9	126	39	116.9	126	53
Teflubenzuron	9.9	380.9	141.1	76	57	158.2	76	23
Thidiazuron	4.1	221.1	102.0	71	19	128	71	25
Tiadinil	5.8	267.9	100.9	61	27	101.1	51	27
Triazophos	5.9	314.1	162.2	71	27	119.2	71	47

The mass spectra for all of the pesticides were obtained using the positive ion mode. R.T., retention time; D.P., declustering potential; and C.E., collision energies.

2.2. Sample Preparation

The QuEChERS extraction procedure was performed as follows: sample (10 mL) was placed in a 50-mL centrifuge tube, and 5.0 mL of acetonitrile was added as the extraction solvent. Triphenyl phosphate (1.0 μg mL^{-1}) was spiked directly into the centrifuge tube as the internal standard. Extraction was performed using the QuEChERS EN extraction kit containing 4.0 g of $MgSO_4$, 1.0 g of sodium chloride, 1.0 g of sodium citrate dehydrate, and 0.5 g of sodium hydrogen citrate sesquihydrate. The centrifuge tube was shaken for 1.0 min, charged with the QuEChERS EN extraction kit, shaken vigorously for 10 min, and centrifuged at 4000× *g* for 10 min at 4 °C.

Various dispersive solid-phase extraction clean-up methods were applied for analyte extraction. The analyte recovery by various clean-up procedures at spiked levels of 10, 50, and 100 μg L^{-1} was evaluated. The following clean-up procedures were used: Clean-up I (50 mg C_{18}, 50 mg PSA, and 150 mg $MgSO_4$); Clean-up II (50 mg C_{18}, 50 mg PSA, 50 mg GCB, and 150 mg $MgSO_4$); Clean-up III (50 mg GCB, 50 mg PSA, and 150 mg $MgSO_4$); Clean-up IV (50 mg Z-Sep, 50 mg PSA, and 150 mg $MgSO_4$); and Clean-up V (7.5 mg ENVI-Carb, 25 mg PSA, and 150 mg $MgSO_4$). A 1-mL aliquot of the extract was transferred to a 2-mL tube and each tube was strongly vortexed for 1 min and then centrifuged at 4000× *g* for 5 min at 4 °C. A 0.5-mL aliquot of the upper layer was transferred to a glass tube and the solvent was evaporated by purging with N_2 gas. After reconstituting with 0.1 mL of acetonitrile, the extract was filtered through a syringe with a 0.22-μm nylon membrane filter and transferred into an autosampler vial for analysis by LC-MS/MS.

2.3. LC-MS/MS

Pesticides were analyzed by using an Agilent LC 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisted of a 4000 QTRAP mass spectrometer coupled with a turbo ion-spray ionization source (AB SCIEX, Foster City, CA, USA). Chromatographic separation was carried out at 40 °C with an Zorbax Eclipse Plus C_{18} column (2.1 × 100 mm, 3.5 μm) supplied by Agilent (Santa Clara, CA, USA). The separation was performed with a mobile phase A (5.0 mM ammonium acetate and 0.1 vol % formic acid in water) and mobile phase B (5.0 mM ammonium acetate and 0.1 vol % formic acid in methanol), at a flow rate 0.4 mL/min; the injection volume was 2.0 μL. A linear gradient was performed as follows: 0–8 min, 60% A; 8–1.5 min, 40% A; 1.5–2.5 min, 30% A; 2.5–9 min 20% A; 9 to 12 min 0% A; 12–15 min, 95% A. The mass analysis using the positive ESI mode was operated in the scheduled multiple reaction monitoring (MRM) mode with the following parameters: curtain gas (30 psi), ion-source gas 1 (50 psi), ion-source gas 2 (55 psi), source temperature (400 °C), and ion-spray voltage, which was set at 5500 V. Table 1 summarized the MRM transitions for each pesticide.

2.4. Method Validation and Matrix Effects

Method validation was carried out according to the European Commission SANTE/12682/2019 [17] guidelines for linearity, matrix effects (MEs), limits of detection (LODs), limits of quantification (LOQs), accuracy (recovery percentage), and precision (relative standard deviation, RSD%). The linearity was determined by analyzing matrix-matched calibration curves with spiked blank samples at concentrations of 5, 10, 20, 50, and 100 μg L^{-1}.

The accuracy and precision were determined by analyzing blank samples spiked with standard solutions at three concentrations: 10, 50, and 100 μg L^{-1}. Repeatability and reproducibility were expressed as intra-day (same day) and inter-day (three different days) RSDs%, respectively, by assaying at least six replicates. LODs and LOQs were determined from five independently spiked concentrations of pesticide (5, 10, 20, 50, and 100 μg L^{-1}). LODs and LOQs were calculated based on standard deviations of response and slope, and are expressed as: LOD = 3.3 σ/s, LOQ = 10 σ/s (where σ = standard deviation of the response, s = slope of the matrix-matched calibration curve).

Additionally, as described in SANTE/12682/2019, matrix-matched standards were used to compensate for MEs. Matrix-matched calibration curves were used to overcome quantification accuracy problems. MEs (%ME) were evaluated by comparing the slopes of the calibration curves in acetonitrile with the matrix-matched calibration curves in the extracts using the following equation: % ME = ((slope of calibration curve in the extract/slope of calibration curve in solvent)−1) × 100 [8].

3. Results and Discussion

3.1. Optimization of LC-MS/MS Conditions

The conditions for the ionization and fragmentation of 55 pesticides were established prior to method validation. The conditions for the quantitative and qualitative analyses of the target pesticides were determined by MRM on the basis of retention times and qualitative and quantitative ion abundances. The target pesticides had previously been successfully quantified and qualified in the positive ESI mode [18,19] under the optimized LC-MS/MS conditions. The MRM transitions were confirmed through direct infusion with a syringe pump at a flow rate of 10 μL min^{-1}. The most intense product ion was chosen as the quantifier ion and the second most selective as the qualifier ion. The optimized LC-MS/MS conditions that afforded the best MRM transitions, including retention times, quantifier and qualifier ion transitions, declustering potentials, and collision energies, are listed in Table 1. The mobile phases used in this study were composed of water (containing 0.1% formic acid and 5 mM ammonium acetate) and methanol (containing 0.1% formic acid); satisfactory separation and resolution were achieved for all pesticides. The analyte peaks eluted separately and exhibited good shapes at a spiked level of 100 μg L^{-1} in an agricultural water sample (Figure 1).

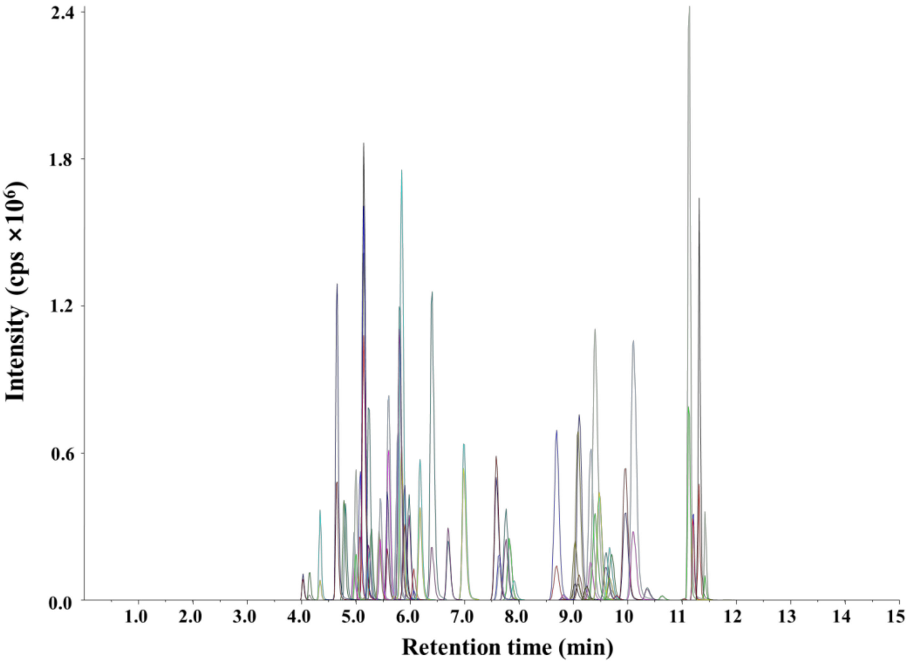

Figure 1. Multiple reaction monitoring chromatogram of 55 pesticides under the optimized LC–MS/MS conditions.

3.2. Clean-Up Sorbent Determination

Since its introduction in 2003 by Anastassiades, QuEChERS has been modified and optimized by many researchers [11,20–22]. Acetonitrile is commonly selected as the extraction solvent for multi-residue pesticide analysis because of its high eluent strength and versatility for both liquid chromatography- and gas chromatography-amenable pesticides [5,11]. In the partitioning process, $MgSO_4$ and NaCl (4:1) are mainly used as salts, resulting in high recovery and fewer co-extractives [11]. However, the sorbents used to remove the co-extractives during the extraction process require appropriate modification and effective approaches because of the presence of various matrices (lipids, flavonoids, etc.) in the samples [11,16]. In this study, the recovery of the sorbents commonly used in the QuEChERS method was evaluated for the analysis of multi-residue pesticides in the samples that are widely exposed in nature, such as agricultural water [10]. The PSA sorbent is a weak anion-exchange agent that removes co-extractives such as sugars and fatty acids [16]. The GCB sorbent helps to remove pigments such as carotenoids and chlorophyll as well as steroids [9,11], while the octadecyl (C_{18}) reverse phase clean-up sorbent retains fats, vitamins, and minerals based on van der Waals interactions or dispersion [11,21].

In a preliminary study, universal sorbents, including PSA, GCB, and C_{18} were used to remove the co-extractives (Figure 2). Almost all pesticides exhibited low recoveries (<70%) when GCB was used as the clean-up sorbent with C_{18} and PSA (Clean-up II), and some pesticides showed low recovery in the absence of GCB (Clean-up I). The low recovery observed for Clean-ups I and II is attributed to the disadvantages of the GCB sorbent, i.e., its high affinity for planar compounds, and the limitations of the PSA sorbent, which is incapable of removing excessive interferents in a complex sample [9,11]. According to Tolosa et al. [6], the GCB sorbent affects pesticide stability through three hydrolysis mechanisms; non-catalyzed hydrolysis by residual water, hydrolysis catalyzed by the chemical heterogeneity of the GCB surface, and chemisorption by surface-active sites.

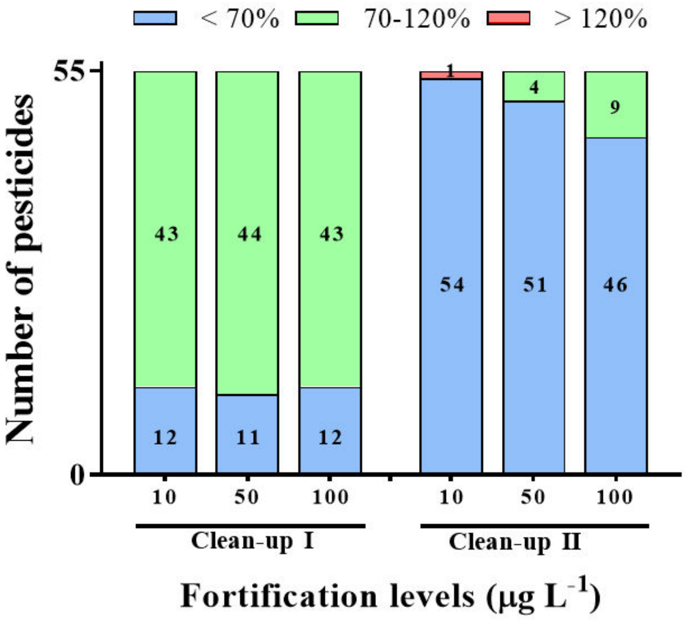

Figure 2. The recoveries of different clean-up procedures for LC-MS/MS at the spiked level of 10, 50, and 100 μg L^{-1}. The different clean-up procedures used were as follows: Clean-up I (50 mg C_{18}, 50 mg PSA, and 150 mg $MgSO_4$); Clean-up II (50 mg C_{18}, 50 mg PSA, 50 mg GCB, and 150 mg $MgSO_4$).

As shown in Figure 3, when Clean-up III (GCB/PSA/$MgSO_4$) was used as the clean-up procedure, all 55 pesticides showed recoveries of less than 70% at all fortification levels. Additional carbon-based sorbents were studied in order to overcome the low recovery issues associated with the Clean-up I and II methods. Z-Sep is a sorbent composed of C_{18} coated with ZrO_2 and has the properties of an amphoteric oxide. As the ZrO_2:C_{18} ratio is 2:5, Z-Sep is expected to exhibit properties identical to those of the C_{18} sorbent [15]. Figure 3 shows that the pesticide recovery achieved for Clean-up IV (Z-Sep/PSA/$MgSO_4$) is similar to that for Clean-up I. While the Z-Sep sorbent effectively removed matrices that contain carboxylic acid groups, such as proteins, its strong Lewis-acidic properties result in the very slow desorption of basic substances [15]. Therefore, we investigate that clean-up with Z-Sep would not satisfy the desired pesticide-recovery criteria.

Figure 3 reveals that Clean-up V (ENVI-Carb/PSA/$MgSO_4$) delivered a recovery of >93.9% during the multi-residue pesticide analysis. We believe that the characteristics of ENVI-Carb are attributable for the highest recovery for Clean-up V. The two-dimensional layered graphite structure endows ENVI-Carb with a large surface area, makes it weakly adsorptive, and does not require the use an analyte to diffuse into pores [8,23]. For this reason, ENVI-Carb does not require a washing step, such as the dichloromethane washing step used for silica cartridge, which reduces the sample-preparation time [23]. In principle, ENVI-Carb interacts in a dispersive manner with non-polar molecules, such as aromatic sorbates, on its surface due to the presence of hybridized π-electrons [24–26]. Hence, the ENVI-Carb sorbent effectively removes trace compounds such as polyphenols and pigments [8,26]. Zhang et al. [26] reported that ENVI-Carb effectively reduces the color of sewage sludge and exhibits low matrix effects. Therefore, we conclude that ENVI-Carb is an effective sorbent for the analysis of multi-residue pesticides in environmental samples.

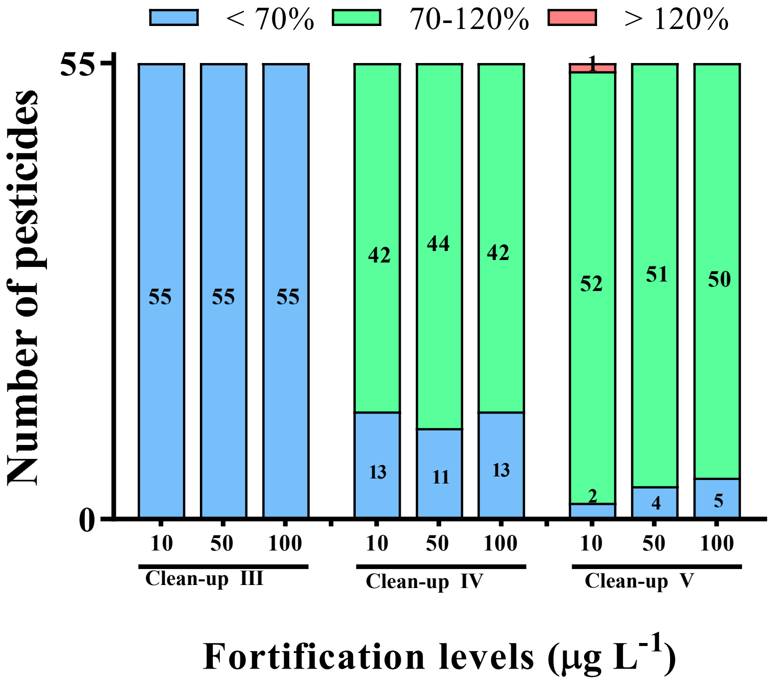

Figure 3. The recoveries of different clean-up procedures for LC-MS/MS at the spiked level of 10, 50, and 100 µg L^{-1}. The different clean-up procedures used were as follows: Clean-up III (50 mg GCB, 50 mg PSA, and 150 mg $MgSO_4$); Clean-up IV (50 mg Z-Sep, 50 mg PSA, and 150 mg $MgSO_4$); Clean-up V (7.5 mg ENVI-Carb, 25 mg PSA, and 150 mg $MgSO_4$).

3.3. Method Validation

The clean-up procedure was validated under the optimized combined-sorbent conditions. The values of several validation parameters, namely, linearity, accuracy, precision, LOD, and LOQ, were determined in order to validate QuEChERS extraction using the Clean-up V method. Linear calibration curves were constructed by the matrix-matched standard calibration method using concentrations of 5, 10, 20, 50, and 100 µg L^{-1} in blank extracts of agricultural water. As summarized in Table 2, satisfactory coefficients of determination (R^2 = 0.9991–0.9999) were obtained for the 55 pesticides. The LOD values of individual pesticides were within 0.02–3.0 µg L^{-1}, which were calculated as having a signal to noise ratios of 3:1 from the chromatograms.

Moreover, the LOQ levels (0.1–9.9 µg L^{-1}) were obtained, with the 55 pesticides exhibiting satisfactory recovery and precision. The recovery of the 55 pesticides ranged between 20.5 and 121.7% and was determined at three fortification levels (10, 50, and 100 µg L^{-1}). However, among the tested pesticides, the MRLs are not currently set for agricultural water in Korea. Thus, we applied the uniform MRL (0.01 mg kg^{-1} or less) to all tested pesticides. The low recovery of pyrazolate (29.1%) and thidiazuron (59.2%) indicates that their levels in agricultural water can only be qualitatively determined, rather than quantitatively. The RSDs for repeatability and reproducibility for all 55 pesticides were found to be <14.8% and 15.8%, respectively. It was also possible to separate the 55 target components to satisfactory levels.

Table 2. Method validation data of selected 55 pesticides in water using Clean-up V (7.5 mg ENVI-Carb, 25 mg PSA, and 150 mg $MgSO_4$) with LC-MS/MS. Range of pesticides concentration between 0.1 to 100 (μg L^{-1}).

Pesticides	**Linearity (R^2)**	**LOD (μg L^{-1})**	**LOQ (μg L^{-1})**	**Recovery (%)**			**Repeatability, %RSD**			**Reproducibility, %RSD**		
				10	**50**	**100**	**10**	**50**	**100**	**10**	**50**	**100**
				μg L^{-1}			**μg L^{-1}**			**μg L^{-1}**		
Amisulbrom	0.9998	3.0	9.9	96.9	78.1	73.7	7.8	7.6	3.8	7.2	9.2	10.4
Azinphos-methyl	1.0000	0.6	1.8	110.2	95.8	92.5	10.9	9.6	3.5	11.8	9.7	4.5
Bensulfuron-methyl	1.0000	0.2	0.6	86.0	77.0	70.1	2.4	11.4	0.8	2.6	8.7	4.3
Benzobicyclon	0.9999	0.1	0.3	68.5	62.1	74.6	13.1	8.8	6.1	13.4	10.6	9.3
Boscalid	1.0000	0.6	2.1	104.3	94.0	91.3	12.2	11.6	9.4	12.4	12.9	7.2
Chlorpyrifos	0.9991	1.0	3.4	103.3	101.2	87.1	2.2	14.4	2.9	3.6	10.6	3.3
Cyclosulfamuron	0.9994	0.02	0.1	105.0	97.7	80.7	11.5	12.9	11.7	9.2	10.8	8.2
Diethofencarb	1.0000	0.2	0.6	118.9	99.5	92.7	5.4	9.7	2.9	3.6	7.2	3.4
Diflubenzuron	1.0000	0.5	1.6	99.6	86.1	80.2	8.9	9.1	2.6	7.8	7.8	7.0
Diuron	0.9998	0.3	1.0	119.3	96.1	95.0	6.8	6.3	6.8	6.1	5.5	4.4
Etoxazole	0.9994	0.2	0.5	108.8	92.4	87.1	4.2	6.9	3.9	3.4	4.7	2.9
Etrimfos	1.0000	0.1	0.2	108.3	99.9	83.5	2.2	7.4	6.1	2.7	7.1	4.8
Fenazaquin	0.9996	2.1	6.9	112.3	88.0	70.0	3.6	6.8	1.8	8.0	4.7	4.6
Fenbuconazole	1.0000	0.5	1.6	108.0	95.7	89.8	10.1	10.0	8.4	8.5	7.6	5.9
Fenhexamid	0.9999	0.6	1.9	102.7	88.6	86.0	3.6	7.9	10.5	9.4	12.1	7.3
Fenoxaprop-ethyl	0.9999	0.4	1.4	108.5	94.4	90.7	5.5	7.5	2.6	5.8	4.9	5.0
Fenpyroximate	0.9999	0.1	0.5	97.0	84.0	78.1	2.6	8.7	0.7	3.1	9.8	4.7
Fludioxonil	0.9999	0.1	0.5	117.5	99.7	88.2	11.3	9.1	11.4	9.4	9.1	8.0
Flufenoxuron	1.0000	0.2	0.8	99.2	90.1	85.0	6.5	11.9	6.5	4.8	12.9	4.9
Fluxapyroxad	0.9990	0.5	1.8	115.9	98.8	95.9	8.6	8.9	5.1	9.3	8.3	3.9
Forchlorfenuron	0.9995	0.7	2.5	70.2	62.1	52.6	9.2	8.3	10.6	5.8	7.3	12.3
Hexaflumuron	0.9999	0.5	1.8	100.0	90.9	89.2	9.7	8.5	8.0	10.6	4.4	11.2
Linuron	0.9998	0.7	2.4	114.6	95.6	94.6	8.3	7.7	6.8	7.0	5.8	4.4
Lufenuron	0.9997	0.7	2.5	103.4	94.2	88.5	6.9	6.2	10.4	5.5	6.8	9.3
Mefenacet	0.9996	0.1	0.3	121.7	99.8	95.9	2.3	8.5	1.0	2.4	6.5	1.3
Mepanipyrim	1.0000	0.4	1.3	110.5	89.0	85.1	6.1	5.1	1.9	12.5	4.5	1.8
Metamifop	1.0000	0.1	0.4	110.3	94.3	86.8	2.3	8.2	0.8	10.0	7.6	4.8
Methabenzthiazuron	0.9996	0.5	1.6	108.9	89.6	87.6	10.4	6.1	5.7	9.3	4.8	4.8
Metrafenone	0.9999	0.2	0.6	107.7	94.0	91.8	5.9	10.9	4.7	5.2	7.4	3.9
Napropamide	0.9999	0.1	0.4	119.9	98.7	91.5	1.9	7.0	4.2	3.6	7.9	3.4

Table 2. *Cont.*

Pesticides	**Linearity (R^2)**	**LOD (μg L^{-1})**	**LOQ (μg L^{-1})**	**Recovery (%)**			**Repeatability, %RSD**			**Reproducibility, %RSD**		
				10	**50**	**100**	**10**	**50**	**100**	**10**	**50**	**100**
				μg L^{-1}			**μg L^{-1}**			**μg L^{-1}**		
Novaluron	0.9998	0.5	1.8	113.4	94.2	96.4	14.8	11.7	9.3	10.2	7.6	9.0
Penoxsulam	0.9998	0.6	2.2	84.6	74.0	72.0	10.2	12.0	13.4	10.7	15.8	9.4
Pirimiphos-methyl	1.0000	0.1	0.3	110.5	96.8	91.1	4.6	7.5	6.4	3.9	4.8	4.6
Probenazole	0.9995	1.7	5.6	111.8	84.9	85.3	3.3	5.2	9.1	5.6	7.0	7.1
Propanil	0.9999	0.9	3.0	102.3	86.9	90.3	8.3	9.5	9.4	12.0	10.3	10.4
Propaquizafop	0.9996	0.1	0.3	101.9	90.6	83.1	4.9	9.3	11.6	8.8	5.4	8.7
Pyraclostrobin	0.9997	0.2	0.6	111.3	92.6	81.7	2.5	7.5	0.7	9.8	1.0	5.3
Pyrazolate	0.9994	1.2	4.0	38.9	20.5	27.9	8.1	11.5	12.4	11.6	12.6	13.5
Pyrazophos	1.0000	0.2	0.7	103.9	92.7	85.8	6.0	9.6	5.5	8.4	7.5	7.3
Pyribenzoxim	0.9999	0.4	1.3	116.1	99.1	94.0	4.9	10.0	2.6	6.3	7.4	2.8
Pyriftalid	0.9994	0.1	0.2	116.7	99.4	94.0	4.6	5.8	1.9	6.7	5.9	5.3
Pyrimethanil	0.9998	0.6	2.0	113.5	87.4	87.0	9.1	3.6	3.3	10.2	11.9	3.3
Pyrimidifen	0.9999	0.3	0.8	100.0	85.4	75.1	2.1	5.5	2.1	3.7	1.4	12.0
Pyriproxyfen	1.0000	0.1	0.4	116.8	99.1	93.8	1.3	8.2	1.2	2.0	5.2	1.4
Quinalphos	0.9998	0.0	0.0	110.0	94.1	87.6	7.1	11.7	3.4	6.5	9.0	2.3
Quinoclamine	1.0000	0.7	2.4	104.2	80.9	81.6	10.5	9.9	2.3	8.2	7.7	11.1
Quizalofop-ethyl	1.0000	0.2	0.8	108.4	92.6	86.6	5.7	7.0	4.4	6.2	4.6	4.7
Sethoxydim	1.0000	1.8	5.9	101.8	86.7	82.0	6.9	9.3	5.5	7.9	8.7	5.7
Spinetoram(J)	0.9999	0.04	0.1	108.3	92.2	85.9	2.1	10.0	1.1	5.0	14.5	3.1
Spinetoram(L)	0.9998	0.3	0.9	107.1	90.2	84.2	2.7	12.1	2.7	5.5	14.5	3.6
Tebufenpyrad	1.0000	0.3	1.1	108.6	96.2	94.0	13.3	12.5	12.1	13.5	11.6	7.7
Teflubenzuron	0.9999	0.6	1.9	89.6	85.2	83.1	9.8	9.9	9.2	11.6	2.5	11.1
Thidiazuron	0.9999	0.6	1.9	74.2	55.3	48.1	4.1	3.6	8.6	6.1	2.2	9.6
Tiadinil	1.0000	2.6	8.6	105.5	89.9	82.9	1.4	11.9	11.7	7.4	12.6	11.3
Triazophos	0.9993	0.1	0.2	114.1	100.0	95.3	3.8	7.1	0.7	4.7	4.9	1.8

QuEChERS extraction was carried out using the EN extraction kit, and clean-up was performed using Clean-up V. LOD, limits of detection; LOQ, limits of quantification; RSD, relative standard deviation.

3.4. Matrix Effects

Matrix effects (MEs) are one of the main aspects that must be addressed when evaluating a multi-residue method for pesticide analysis. The slope of a calibration curve is considerably influenced by matrix components that are not removed during clean-up [27]. Minimizing MEs improves chromatographic selectivity, and dilution is an effective approach for this [18,19]. In this study, MEs (%ME) were determined on the basis of ion suppression and/or enhancement [19]. Reagent and matrix standards for each concentration (10, 50, and 100 µg L^{-1}) were analyzed using Clean-up V. As shown in Figure 4, all compounds showed slight ion suppression, with MEs ranging from −47.3% to 9.5% (average: −11.9%), with pyrazolate exhibiting the highest ME (−47.3%). Other pesticides that showed MEs < 20% include fenbuconazole (−21.2%), fenhexamid (−23.8%), forchlorfenuron (−29.1%), and thidiazuron (−25.5%). The recoveries of these five pesticides were mostly less than 70%, except one or two fortification levels. Ion suppression and/or enhancement through MEs depend on several factors, including sample preparation procedure, sample matrix, and physicochemical properties of the pesticide [28,29]. A few alternative techniques have been suggested to compensate for MEs. Among them, external matrix-matched calibration using blank samples spiked with standard solutions is commonly employed because of its low cost and lack of complexity [29,30].

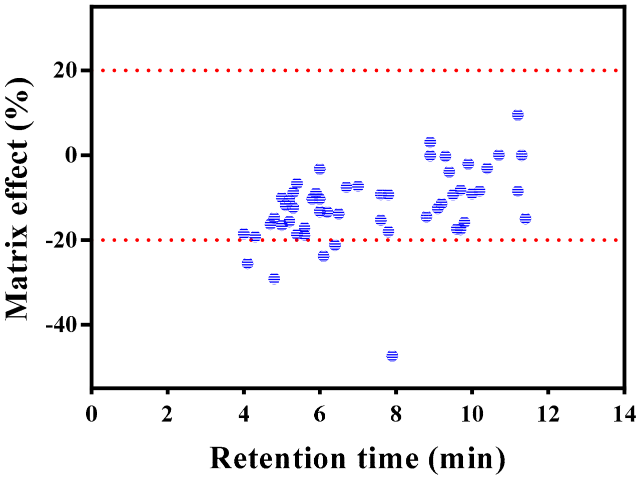

Figure 4. Matrix effects of 55 pesticides obtained by Clean-up V (contained ENVI-Carb) procedure.

4. Conclusions

We developed a clean-up procedure that uses a combined sorbent for the LC-MS/MS determination of multi-residue pesticides in agricultural water. The GCB sorbent is commonly used to effectively remove pigments, such as chlorophyll, in an agricultural water sample; however, GCB exhibits low recovery for multi-residue pesticides. As an alternative, we selected ENVI-Carb and showed that it positively influenced the clean-up process for extracts from agricultural water samples. The combined use of PSA and ENVI-Carb facilitated good linearity, precision, and accuracy during the multi-residue pesticide analysis. This approach is suitable for quantitative analysis and for detection of multi-residue pesticides in agricultural water.

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