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Enhancement of Tricyclazole Analysis Efficiency in Rice Samples Using an Improved QuEChERS and Its Application in Residue: A Study from Unmanned Arial Spraying

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Abstract: Enhancements to the analytical method for the determination of tricyclazole in rice samples have been applied to monitor residues during unmanned aerial spraying. The acetonitrile extraction technique QuEChERS was improved by the incorporation of ethyl acetate and 0.1% formic acid, which significantly elevated the recovery rates. Furthermore, the purification process was refined by integrating both primary–secondary amine (PSA) and C18 in the dSPE method, achieving a substantial improvement in reducing matrix effects (MEs) and increasing recovery efficiency. The optimized method demonstrated an impressive % ME value at −3.1%, with a limit of quantitation (LOQ) established at 0.01 mg/kg, and recovery rates between 94.7 and 95.6% at 0.01, 0.1, and 2 mg/kg. Using two types of adjuvants (stickers) during multi-copter spraying markedly improved the initial tricyclazole deposition on rice panicles, with residue levels initially increasing from 0.35 mg/kg to between 0.68 and 1.60 mg/kg. Residues in hulled rice at harvest (10 days post-application) remained well below the maximum residue limit (MRL) of 0.7 mg/kg, ranging from 0.02 to 0.11 mg/kg, thus affirming the safety and efficacy of adjuvants in residue management.

Keywords: rice; LC-MS/MS; QuEChERS; tricyclazole; unmanned arial vehicle (UAV); adjuvant

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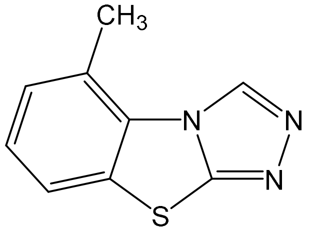
1. Introduction

Tricyclazole, with its IUPAC name 5-methyl-1,2,4-triazolo[3,4-b]benzothiazole (Figure 1), is a famous fungicidal agent effective against rice blast caused by *Magnaporthe oryzae* (*Pyricularia oryzae*). It was developed over 35 years ago as a melanin biosynthesis inhibitor, and specifically hinders the melanin production in fungus's appressoria, essential for the host penetration [1]. By inhibiting the conversion of 1,3,6,8-tetrahydroxynaphthalene to scytalone and 1,3,8-trihydroxynaphthalene to vermelone, tricyclazole weakens the mechanical strength of the appressoria, thus impairing the fungus's ability to infect the host [2,3]. This specific interference with the pathogen's developmental cycle presents a strategic means of mitigating rice blast outbreaks.



5-methylbenzo[4,5]thiazolo[2,3-*c*][1,2,4]triazole

Figure 1. Structural formulas and IUPAC names of the active ingredient tricyclazole.

Rice is one of the most important staple foods worldwide, feeding more than half of the global population, particularly in Asia [4]. As a primary source of sustenance, ensuring the safety and quality of rice is paramount for food security. The high consumption rates underscore the need for rigorous monitoring of any potential contaminants that could impact public health. The use of pesticides in agriculture is essential for controlling pests and diseases, yet their residues can pose significant risks to human health and the environment [5,6].

The inherent characteristics of paddy field soil, particularly its waterlogged condition, make conventional methods of pesticide application challenging and cumbersome. In response to these limitations, unmanned aerial vehicles (UAV), specifically multi-copters (drones), have been introduced as a viable alternative for pesticide dispersal in rice cultivation [7,8]. One of the primary advantages of arial spraying is its capability to cover large areas swiftly, being able to treat up to one hectare in less than 10 min. This rapid application significantly reduces labor costs and exposure risks to workers who would otherwise be applying pesticides manually.

One concern with arial spraying technology is the potential for off-target drift, where pesticides may spread from the intended area to surrounding environments [9]. This can lead to unintended exposure of wildlife and non-target plant species to chemicals, raising environmental and ecological concerns. To mitigate the risks associated with pesticide drift, the use of adjuvants such as stickers has been recommended. Stickers increase the adherence of pesticide droplets to the plant surfaces and reduce the dynamic surface tension, thus reducing the amount of chemical that drifts away from the target site [7,10]. While this improves the efficacy of the pesticide application, it also increases the concentration of chemicals on the crop [11], which necessitates rigorous monitoring to ensure that residue levels remain within safe limits for human consumption.

To achieve accurate monitoring of pesticide residues such as tricyclazole, there is a crucial need for developing analysis methods specifically tailored to this fungicide. The literature reveals that while methods for detecting tricyclazole in rice samples are commonly incorporated into multiresidue analyses, dedicated methodologies focused solely on tricyclazole are notably absent [12–18]. This lack of specificity can compromise the ruggedness of single-target residue determination. Multiresidue techniques often introduce significant challenges in terms of matrix effects for some target analytes. In liquid chromatography-tandem mass spectrometry (LC-MS/MS), tricyclazole frequently exhibits strong signal suppression [14,18], whereas in gas chromatography-tandem mass spectrometry (GC-MS/MS), it can show pronounced signal enhancement by sample matrices [16]. These matrix effects, which vary even among samples of the same crop variety, can cause the variability and potential inaccuracies in quantifying residues [19]. Consequently, there is a pressing need to reduce the matrix effects to negligible levels.

The objective of this study is to advance the methodology for analyzing tricyclazole residues in rice samples. This includes refining the QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction, partitioning, and purification procedures and assessing the effect of various adjuvants on residue levels through unmanned aerial spraying. The significance of this research lies in its potential to enhance the accuracy and efficiency of tricyclazole residue analysis in rice samples, with the innovative reduction of matrix effects. By improving analytical methods, the study aims to ensure food safety, comply with regulatory standards, and provide insights into effective pesticide application and residue management during unmanned arial spraying. This work is critical for maintaining consumer health, supporting agricultural practices, and fostering environmental sustainability by minimizing unintended pesticide spread and accumulation in the ecosystem.

2. Materials and Methods

2.1. Reagents

Tricyclazole (purity 99.4%) reference standard was purchased from Merck (Darmstadt, Germany). LC-MS grade methanol and formic acid (FA; purity > 99%) were procured from Thermo Fisher Scientific (Waltham, MA, USA), and LC-MS grade water was obtained from Merck. HPLC-grade MeCN and EA were sourced from Samchun Chemical (Seoul, Republic of Korea) and Duksan Pure Chemical (Seoul, Republic of Korea), respectively. The Oasis PRiME HLB cartridge plus light (100 mg) was sourced from Waters Corporation (Milford, MA, USA). QuEChERS extraction Original pouches (magnesium sulfate; $MgSO_4$ [4 g], sodium chloride; NaCl [1 g]) and EN 15662 pouches ($MgSO_4$ [4 g], NaCl [1 g], sodium citrate; $Na_3Citr \cdot 2H_2O$ [1 g], and sodium hydrogen citrate sesquihydrate; $Na_2HCitr \cdot 1.5H_2O$ [0.5 g]), along with dispersive SPE (dSPE) tubes Part No. 5982-5021 (PSA [25 mg] and $MgSO_4$ [150 mg]), 5982-4921 (C18 [25 mg] and $MgSO_4$ [150 mg]), 5982-5121 (PSA [25 mg], C18 [25 mg], and $MgSO_4$ [150 mg]), and 5982-5122 (PSA [50 mg], C18 [50 mg], and $MgSO_4$ [150 mg]) were purchased from Agilent Technologies (Santa Clara, CA, USA).

2.2. Preparation of Standard Solutions and Matrix-Matched Standard Solutions

The tricyclazole reference standard was dissolved in MeCN to prepare a stock solution at a concentration of 1000 mg/L. The solution was then stepwise diluted with MeCN to yield standard solutions with concentrations of 200, 10, 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005, and 0.0025 ng/mL, respectively. To prepare the matrix-matched standard solutions, 300 µL of pesticide-free rice extract was mixed with 300 µL of the standard solution, achieving a 1:1 (*v*/*v*) ratio. The concentrations of these matrix-matched standards, used for plotting the calibration curve, ranged from 1.25–250 ng/mL, which is equivalent to 0.005–1 mg of analytes per kg of the sample.

2.3. LC-MS/MS Instrumental Condition

The pesticide analysis was carried out on an LCMS-8040 triple quadrupole mass spectrometer coupled with a Nexera liquid chromatograph, both sourced from Shimadzu (Kyoto, Japan). For the separation of analytes from interferences, a Cadenza CD-C18 column (3 µm, 2 × 150 mm; Imtakt Corp., Kyoto, Japan) was used. Mobile phase A consisted of 0.1% FA in water, and mobile phase B consisted of 0.1% FA in methanol. The gradient for mobile phase B was initiated at 80% for 0.2 min, then rose to 92% over the next 3.3 min. This was followed by a rapid increase to 98% over 0.1 min and a 2.4 min duration to cleanse the column of impurities. Afterward, the mobile phase was adjusted from 98% back to 80% within 0.1 min and held for another 3.4 min to re-equilibrate the column for the next sample injections. The overall runtime for the analysis was 9.5 min. The column oven temperature was steady at 40 °C, with a flow rate set at 0.2 mL/min and an injection volume of 1 µL.

The tandem mass spectrometer utilized the positive electrospray ionization (ESI+) mode. A pure argon gas (99.999%) was employed for collision-induced dissociation (CID). The temperatures of the desolvation line (DL) and heat block within the ion source were set at 250 °C and 400 °C, respectively. The flow rates of the drying gas and nebulizing gas were 15 L/min and 3 L/min, respectively. Data analysis was conducted using the Shimadzu LabSolutions software (version 5.60 SP2).

For the trace analysis via multiple reaction monitoring (MRM), the optimal transition patterns for tricyclazole were established as follows. Firstly, a mass-to-charge ratio (m/z) of the precursor ion was determined using a full scan analysis. Subsequently, the fragmentation of the precursor ion at various collision energies (CE) was verified through a product ion scan. Two MRM transitions with high selectivity and sensitivity were chosen as the quantifier and qualifier, respectively.

2.4. Comparison of Extraction Solvents and Partitioning Conditions

A water saturated sample (5 g) was spiked with 50 µL of a 10 mg/L standard solution, resulting in a pesticide concentration of 0.1 mg/kg within the sample. Subsequently, three different solvents were used for extraction: MeCN, MeCN/EA (1:1, *v*/*v*), and EA, with 10 mL of each solvent added, respectively. The extracted solution was then partitioned using either QuEChERS Original salts (4 g of $MgSO_4$ and 1 g of NaCl) or EN 15662 salts (4 g of $MgSO_4$, 1 g of NaCl, 1 g of $Na_3Citr{\cdot}2H_2O$, and 0.5 of $Na_2HCitr{\cdot}1.5H_2O$). Without further purification, 300 μL of the organic solvent phase was taken and mixed with 300 μL of MeCN for matrix-matching. The final solution (1 μL) was injected into the mass spectrometer, and the signal (area) of the chromatographic peak was compared to the signal intensity of a matrix-matched standard with the corresponding concentration to calculate the recovery rate (n = 3). Statistical analysis of the recovery rates was conducted using the R software (version 4.3.1) to determine significance among the tested groups. For the extraction/partitioning combinations that exhibited the best recovery rates, extraction efficiencies were further assessed using solvents supplemented with 0.1% FA.

2.5. Comparison of Sample Purification Conditions

A 5 g sample was moistened and spiked with the pesticide working solution to achieve a concentration of 0.1 mg/kg. The sample was subsequently extracted using 10 mL of a 1:1 (v/v) mixture of MeCN and EA, containing 0.1% formic acid. The extraction was then partitioned using the Original QuEChERS salts. The following dSPE methods were employed for the purification of the extracts: (1) PSA purification, utilizing 25 mg of PSA and 150 mg of $MgSO_4$; (2) C18 purification, using 25 mg C18 and 150 mg of $MgSO_4$; (3) a combined PSA and C18 purification, with 25 mg of each PSA and C18, and 150 mg of $MgSO_4$; (4) a larger combined PSA and C18 purification, with 50 mg of each PSA and C18, and 150 mg of $MgSO_4$. For each purification method, 1 mL of the supernatant was transferred into a dSPE tube, mixed, and subsequently, the purified extract was matrix-matched with MeCN in a 1:1 ratio. The recovery rates and matrix effects were compared using three replicates (n = 3).

2.6. Established Preparation Conditions

A quantity of 5 g of finely ground brown rice was placed into a 50 mL centrifuge tube, followed by the addition of 7 mL of water for moistening over a period of 15 min. Subsequently, 10 mL of MeCN/EA (1:1, v/v) solution containing 0.1% FA was introduced. The sample was subjected to extraction using a Geno/Grinder (1600 Mini-G, SPEX SamplePrep, Metuchen, NJ, USA) at a speed of 1300 rpm for 2 min. This process was followed by the addition of 4 g of $MgSO_4$ and 1 g of NaCl, and the mixture was agitated again at 1300 rpm for 1 min. Centrifugation was then performed using a centrifuge (M15R, Hanil Scientific, Gimpo, Republic of Korea) at 3500 rpm for 5 min to facilitate phase separation. A volume of 1 mL from the supernatant was transferred to a dSPE tube containing PSA (25 mg), C18 (25 mg), and $MgSO_4$ (150 mg), and mixed for a duration of 1 min. Further centrifugation was carried out using a microcentrifuge (1248, Labogene, Lillerød, Denmark) at 13,000 rpm for 5 min to precipitate the solids. The supernatant, in a volume of 300 μL, was then matrix-matched with 300 μL of MeCN, and a 1 μL aliquot was analyzed using LC-MS/MS for the detection and quantification of tricyclazole in the sample.

2.7. Analytical Method Validation

The limit of quantitation (LOQ) for the established analytical method was determined by identifying the minimum concentration at which the signal-to-noise ratio (S/N) of the matrix-matched standard solution exceeded 10. The linearity of the calibration curve was assessed using the correlation coefficient (r^2) derived from a first-order regression analysis. For conducting recovery tests (n = 3), standard solutions with concentrations of 1 mg/L and 10 mg/L were separately spiked into 5 g samples to achieve analyte concentrations of 0.01 mg/kg and 0.1 mg/kg, respectively. Pesticides were extracted using the predefined preparation method and then matrix-matched with MeCN in a 1:1 volumetric ratio. To validate the quantitation of analytes exceeding the range of the calibration curve, a 200 mg/L standard solution was spiked into a 5 g sample to reach a concentration of 2 mg/kg. The extract solution was diluted by mixing 30 μL of it with 270 μL of a pesticide-free sample extract and 300 μL of MeCN, followed by the calculation of the recovery rate (n = 3). The matrix effect was quantified by comparing the slope of the calibration curves for the matrix-matched standard solution (*a*) to that of the solvent standard solution (*b*). This value, expressed as a percentage (ME, %), was calculated using Equation (1):

$$\text{ME}\ (\%) = ((a - b)/b) \times 100 \tag{1}$$

2.8. Unmanned Arial Spraying in Paddy Fields

The field trial was conducted in a paddy field located in Daeya-myeon, Gunsan City, Jeollabuk-do, where the rice variety Shindongjin was cultivated. The pesticide application commenced on 22 July 2022, adhering to the safety usage standards which dictated three treatments. The test agrochemical was Nonsarang (Kyungnong, Deagu, Republic of Korea), a commercial formulation containing 8% tricyclazole and 15% ferimzone (Suspension concentrate; SC), which was diluted at an 8-fold rate for spraying. Three treatment plots were established: Plot A received the pesticide product alone; Plot B was treated with agrochemicals combined with an adjuvant, Cares (Dongbangagro, Seoul, Republic of Korea), which made up 1% of the spray solution; Plot C involved agrochemical combined with a different adjuvant, Gondor (De Sangosse, Agen, France), which constituted 2% of the spray solution; and Plot D was a pesticide untreated control group. Each treatment plot was arranged with a spraying radius of 2 m and spaced 8 m apart for three replicates. To minimize temporal variability in the application of treatments, three multi-copters were deployed simultaneously for plots A–C.

The pesticide was dispensed using a multi-copter model YMR-08 (Yamaha Motor Co., Ltd., Shizuoka, Japan) under the following conditions: wind speed at 0 m/s, ensuring applications were made in the calm of early morning between 7 and 10 A.M. The multi-copters were flown at an altitude of 3 m and a speed of 11 km/h and equipped with XR-11001 nozzles (Spraying Systems Co., Glendale Heights, IL, USA) that operated at a pressure of 40 psi and produced an output velocity of 13 m/s to ensure even distribution of the agrochemicals across the designated field areas.

2.9. Sample Collection

Rice panicles were collected two hours after the final pesticide application on 21 September 2022. Ten days following the last treatment, grains were harvested, then subjected to threshing and milling processes to produce brown rice. All samples were pulverized using a mixer and stored at −20 °C until preparation to prevent degradation of tricyclazole.

3. Results

3.1. Establishment of MRM Conditions for Tricyclazole Detection

The molecular formula of tricyclazole is $C_9H_7N_3S$, and its monoisotopic mass has been precisely determined to be 189.2. When analyzed using LC-MS/MS in the ESI+ ionization mode, it underwent ionization to form an $[M+H]^+$ ion by adding a proton (H^+) as an adduct. This resulted in the precursor ion's m/z value being established at 190.2, indicating an increase of one unit over the monoisotopic mass. The product scan analysis revealed major product ions for tricyclazole at m/z values of 163.1, 136.1, 109.1, and 65.1 (Figure S1), findings that are consistent with those reported in the EURL-DataPool database [20]. A comparison of signal intensities for product ions across various CE facilitated the identification of the optimal CE, with corresponding results presented in Table S1. Based on sensitivity criteria (Figure S2), the product ion at m/z 163.1 showed the highest peak intensity at its optimal CE, making it the chosen quantifier ion. The ion at m/z 136.1 displays the second highest peak intensity; thus, it is selected as the qualifier ion. Using the established MRM conditions, the retention time (t_R) of tricyclazole within the C18 column was determined to be 2.0 min.

3.2. Establishment of Optimal Extraction and Partitioning Conditions

The QuEChERS extraction methodology predominantly employs acetonitrile (MeCN) as its extraction solvent [21]. MeCN, a polar organic solvent, exhibits excellent miscibility with pure water, and recent studies have investigated enhancing the extraction efficacy of pesticides by incorporating EA, a solvent with lower polarity, into the extracts [22,23]. To determine the most effective extraction and partitioning conditions for the residue of tricyclazole in brown rice, this study compared the pesticide recovery rates using three different extraction solvents: MeCN, a 1:1, *v*/*v* mixture of MeCN/EA and EA, and two types of QuEChERS salts (Original and EN 15662), as depicted in Table 1.

Table 1. Recovery rates (n = 3) of tricyclazole depending on extraction solvents and partitioning salts in brown rice. Each method was conducted without purification steps.

No.	Extraction Solvent	Partitioning Salts	Recovery ± sd [1] (%)
1	MeCN	Original [2]	86.7 ± 1.6 a [4]
2	MeCN	EN 15662 [3]	86.4 ± 1.3 a
3	MeCN/EA (1:1, *v*/*v*)	Original	86.6 ± 2.0 a
4	MeCN/EA (1:1, *v*/*v*)	EN 15662	83.9 ± 1.0 ab
5	EA	Original	81.6 ± 0.2 bc
6	EA	EN 15662	78.9 ± 0.7 c

[1] Standard deviation. [2] QuEChERS Original salts (4 g of $MgSO_4$ and 1 g of NaCl). [3] EN 15662 salts (4 g of $MgSO_4$, 1 g of NaCl, 1 g of $Na_3Citr{\cdot}2H_2O$, and 0.5 of $Na_2HCitr{\cdot}1.5H_2O$). [4] Significant differences ($p < 0.05$) are indicated by differing letters (a–c).

MeCN yielded the highest recovery rates, especially when used with Original QuEChERS salts. EA alone resulted in the lowest recovery rates. The type of partitioning salt also impacted the results, with Original QuEChERS salts generally outperforming EN 15662 salts across all solvent combinations. This could indicate that the composition of the EN 15662 salts may not be as effective for tricyclazole recovery in the conditions tested. The observed trends in brown rice align with findings from biological matrices such as serum and urine [24,25], where salts without pH buffers have been reported to deliver better recovery rates than buffered salts in multiresidue analysis. This consistency across different matrices underscores the reliability of original QuEChERS salts in diverse analytical contexts. When comparing solvents, a mix of MeCN/EA yielded higher recovery rates than using EA alone, suggesting that the polarity of the solvent may play a significant role in the efficiency of tricyclazole extraction.

In all conditions, recovery rates fell within the acceptable range of 70–120%. Utilizing analysis of variance (ANOVA), the significance of differences among treatments was confirmed. It indicated that Methods No. 1–3 showed a significant increase in recovery rates compared to Methods No. 4–6 ($p < 0.05$), prompting further comparative analyzes of the extraction efficiencies of methods No. 1–3.

3.3. Impact of FA on Tricyclazole Recovery in QuEChERS Extraction Methods

The recovery rates of tricyclazole were evaluated using three different extraction methods with the inclusion of FA in the extraction solvent, as summarized in Table S2. The addition of 0.1% FA in MeCN combined with Original QuEChERS salts resulted in a slight decrease in recovery rates by 0.5%, as compared to the method not including FA (Tables 1 and S2). When EN 15662 salts were used in conjunction with 0.1% FA in MeCN, a further slight reduction was observed, with a 1.8% decrease in recovery rates. In contrast, the use of a MeCN/EA mixture with Original salts in the presence of 0.1% FA led to a significant increase ($p < 0.05$) in recovery rates, with an average recovery of 90.9% and a 4.3% increase compared to the condition without FA.

These findings indicate that the efficiency of the extraction solvent can be modulated by the presence of FA and the type of partitioning salts used. Notably, the combination of MeCN/EA with Original QuEChERS salts and FA showed an improved recovery rate.

In samples with high sugar content, such as herbal matrices, the addition of FA to the MeCN/EA has been known to enhance the extraction efficiency of analytes [22], suggesting a beneficial strategy for extracting compounds.

3.4. Optimization of Purification Conditions

In the pursuit of optimizing purification conditions, extracts derived from the most efficacious extraction and partitioning methodology for tricyclazole—incorporating 0.1% FA in a MeCN/EA (1:1, *v*/*v*) mixture combined with the Original QuEChERS approach—were further processed. The purification efficiency was evaluated by treating these extracts with principal dSPE sorbents (PSA, C18, and PSA+C18) and PRiME HLB plus light (HLB).

When PSA and C18 were individually used at a quantity of 25 mg (Methods No. 1 and 2), the average recoveries were recorded at 90.8% and 92.1%, respectively. Statistical analysis using ANOVA indicated no significant differences between the two single-sorbent methods (Table 2). HLB (No. 5) was shown to yield a competitive recovery rate of 90.9%, nearly mirroring the performance of single-use PSA, which supports its utility as an effective cleanup method for tricyclazole analysis.

Table 2. Comparison of tricyclazole recovery rates and matrix effects using different dSPE sorbents and HLB cartridge cleanup in brown rice.

No.	Purification Method (Major Sorbents)	Recovery ± sd [1] (%)	Matrix Effect ± sd (%)
1	dSPE (PSA, 25 mg)	90.8 ± 2.1 b [2]	−12.9 ± 2.4 b
2	dSPE (C18, 25 mg)	92.1 ± 1.3 b	−17.0 ± 1.9 b
3	dSPE (PSA, 25 mg and C18, 25 mg)	96.7 ± 1.1 a	−6.1 ± 0.3 a
4	dSPE (PSA, 50 mg and C18, 50 mg)	86.4 ± 1.4 c	−6.2 ± 2.6 a
5	PRiME HLB plus light	90.9 ± 1.6 b	−12.8 ± 0.9 b

[1] Standard deviation. [2] Significant differences ($p < 0.05$) are indicated by differing letters (a–c).

Interestingly, utilizing a combination of PSA and C18, with each at the same dosage (No. 3), afforded the highest recovery of 96.7% ($p < 0.05$). This suggests a synergistic effect, with the dual-sorbent system enhancing the cleanup by effectively reducing matrix interferences more than when either sorbent was used independently. Conversely, doubling the amount of each sorbent to 50 mg each did not enhance the recovery (No. 4); the recovery rate observed was lower at 86.4% for the dSPE. The intended purpose of using sorbents like PSA and C18 is to selectively bind and remove contaminants and impurities from the sample matrix, thereby enhancing the purity of the analyte for subsequent analysis [21,26–28]. However, an excess in sorbent quantity could introduce additional binding sites that remain unoccupied by impurities. This can result in the unintentional adsorption of tricyclazole molecules themselves, diminishing their presence in the final extract measured. The same extraction conditions were applied to ferimzone Z and its isomer E form, which also exhibited a decrease in recovery rates as the quantity of purification sorbent increased, so this further supports the notion [23].

The matrix effects were significantly influenced by the choice of dSPE sorbents and HLB cartridge used during the cleanup in brown rice (Table 2). The combination of PSA and C18 (No. 3 and 4) minimized matrix effects to a notable degree, resulting in values of −6.1% to −6.2%, which indicates a highly efficient cleanup. In contrast, the other sorbents and conditions tested showed varying degrees of matrix interference. This highlights the importance of sorbent selection in reducing matrix effects, which is crucial for accurate quantification in residue analysis.

The matrix effects appeared to be relatively unaffected by the increased amounts of the sorbents PSA and C18, when compared at 25 mg and 50 mg each. Despite the doubling of the sorbent mass, the matrix effect remained comparable, indicating that beyond a certain threshold, additional sorbent does not necessarily correlate with increased interference reduction. The correct balance of sorbent properties is key to achieving optimal cleanup and ensuring the reliability of analytical results in complex sample matrices.

Following extensive testing on recovery and matrix effects, the optimal purification strategy was established, and using 25 mg each of PSA and C18 sorbents (Method No. 3) was selected as the final approach. This method not only offered the highest tricyclazole recovery rate but also effectively minimized matrix effects, ensuring the most reliable cleanup for rice sample matrices.

3.5. Analytical Method Validation of Tricyclazole Detection

The analytical performance of the method for detecting tricyclazole was critically evaluated, focusing on the LOQ, linearity, recovery, and matrix effect (Table 3). The LOQ was determined to be 0.01 mg/kg, which is significantly lower than the maximum residue limit (MRL) of 0.7 mg/kg in Korea (Pesticide MRLs in Food, Ministry of Food and Drug Safety). This indicates that the method possesses adequate sensitivity for detecting tricyclazole levels well below the regulatory limit, ensuring compliance with food safety standards. The method demonstrated excellent linearity, as evidenced by a correlation coefficient (r^2) of 0.9996, which indicates a near-perfect linear relationship between concentration and signal response (Figure S3). This strong linearity is essential for ensuring that quantitative results are accurate and reliable across the scope of concentrations tested.

Table 3. Analytical method validation parameters for tricyclazole detection: limit of quantitation (LOQ), linearity, recovery, and matrix effect (ME).

LOQ (mg/kg)	Linearity (r^2)	Recovery (RSD) (%)			ME (%)
		0.01 mg/kg	0.1 mg/kg	2 mg/kg	
0.01	0.9996	95.2 (7.9)	95.6 (2.8)	94.7 (2.6)	−3.1

Recovery experiments were conducted at three fortification levels: 0.01, 0.1, and 2 mg/kg (Table 3). The recovery rates at these concentrations were consistently excellent, recorded at 95.2%, 95.6%, and 94.7%, respectively. The RSDs were notably low, at 7.9%, 2.8%, and 2.6%, demonstrating the method's precision and reproducibility. Such recovery results provide confidence in the method's robustness and its ability to yield reliable data. Interestingly, the matrix effect (ME) was calculated to be −3.1%, indicating a slight suppression in signal due to matrix components. This suppression is minor and within acceptable limits [19,29], suggesting that the method is relatively free from matrix-induced interferences that could potentially bias the results. It was confirmed that no signal was detected in the chromatogram of control sample at the retention time (Figure 2a), and both the solvent standard and the matrix-matched standard at the same LOQ level showed the same intensity (Figure 2b,c), indicating no influence from the rice matrices.

In previous studies, the matrix effects observed for multiresidue pesticide analysis in rice were significantly higher. For example, using LC-MS/MS, the matrix effects ranged from −74% to −32% (medium-strong suppression), and in the case of GC-MS/MS, the matrix effects were reported to exceed 500% (strong enhancement) [14,16,18]. Such substantial matrix effects can complicate the quantification and detection of pesticide residues, leading to potential inaccuracies in the results. For the precise monitoring of a single active ingredient in agricultural fields, single-residue analysis can offer greater specificity and reduced matrix interference, thus providing more accurate and reliable results.

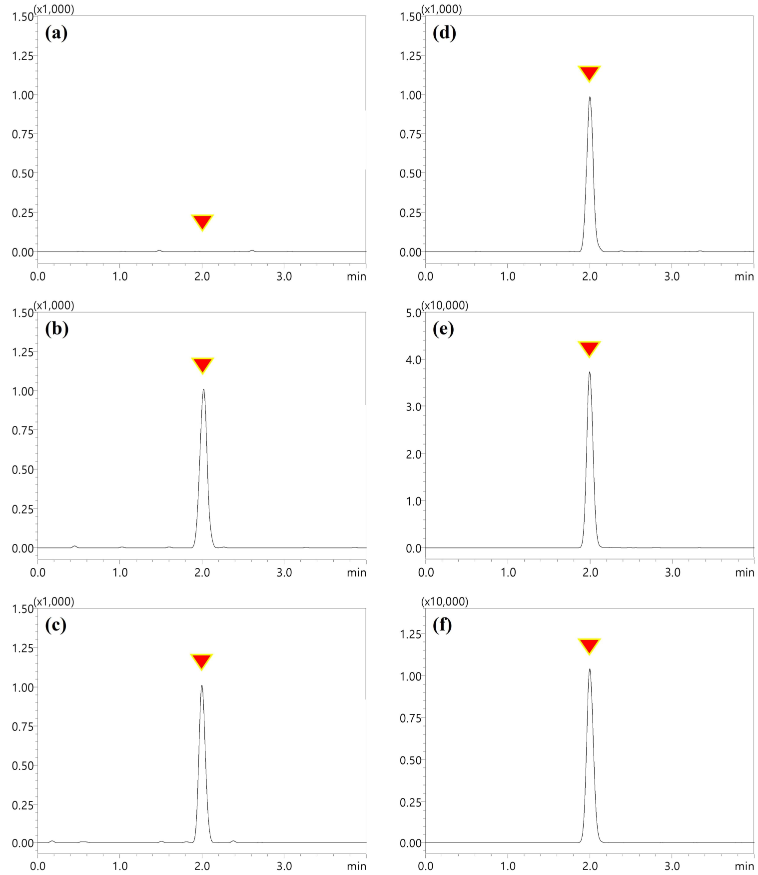


Figure 2. Chromatograms for tricyclazole. The orange triangle symbols indicate the retention time (t_R) at 2.0 min. (**a**) Control sample, (**b**) solvent standard at 0.01 mg/kg, (**c**) matrix-matched standard at 0.01 mg/kg, (**d**) recovery sample at 0.01 mg/kg, (**e**) panicle sample in Plot A, and (**f**) brown rice sample in Plot A.

3.6. Application in Residue Study from Unmanned Arial Spraying

The study successfully applied the established analytical method to the residue trials (Figure 2e,f), allowing for the quantification of tricyclazole residues after unmanned aerial spraying in paddy fields. This application demonstrated the method's robustness and adaptability to real-world conditions.

The findings revealed that the type of adjuvant used significantly affected the residue levels of tricyclazole (Table 4) at 0 DAT. The highest mean residue in rice panicle was observed in Plot C (1.60 mg/kg), where Gondor was used as the adjuvant, while Plot A, with no adjuvant, showed the lowest residue levels (0.35 mg/kg). Plot B, with the adjuvant Cares, exhibited moderate residue levels (0.68 mg/kg). These results underscore the importance of selecting appropriate adjuvants to achieve optimal pesticide efficacy and adherence, which, in turn, influences residue levels. There have been reports indicating that adjuvants can significantly impact droplet size, thereby contributing to the reduction of off-target drift [30,31]. This directly affects the efficiency of pesticide delivery and the residue levels on crops, highlighting the critical role of adjuvants in precision agriculture.

Residual concentrations of tricyclazole in brown rice at 10 DAT were assessed, as illustrated in (Table S3). Plot A, applied according to the established safety usage standards, demonstrated residues below the MRL of 0.7 mg/kg, affirming the effectiveness of the pesticide application while maintaining regulatory compliance. Plots B and C also stayed well within the MRL. The investigation highlighted that while adjuvants increased initial attachment of tricyclazole at 0 DAT, ultimately, at the point of harvest (10 DAT), residue levels remained safely within the established MRL. This suggests that the use of adjuvants can be considered safe, since they do not cause residue levels to exceed regulated limits, and that they potentially contribute to environmental safety by minimizing off-target drift. The major components of Cares and Gondor are natural paraffin and soy lecithin, respectively, both of which are recognized as safe for agricultural use. Thus, their use is considered safe for human health when applied to agricultural pest controls.

Table 4. Residual concentrations of tricyclazole in rice panicles at 0 days after treatment (DAT) following aerial spraying with various adjuvants.

Plot	Adjuvant	Residue (mg/kg)			
		Trial 1	Trial 2	Trial 3	Mean ± sd [1]
A	-	0.27	0.35	0.43	0.35 ± 0.08 c [2]
B	Cares	0.71	0.57	0.78	0.68 ± 0.11 b
C	Gondor	1.45	2.01	1.35	1.60 ± 0.36 a

[1] Standard deviation. [2] Significant differences ($p < 0.05$) are indicated by differing letters (a–c).

4. Conclusions

In this study, the QuEChERS methodology was refined for better tricyclazole detection in rice samples, and the established method was successfully applied to residue study from unmanned aerial spraying. It was discovered that utilizing a mixed solvent of MeCN and EA, containing 0.1% FA, yielded the most efficient extraction and partitioning of tricyclazole. The combined use of PSA and C18 (25 mg each) sorbents during purification maximized recovery rates and reduced matrix effects, though excessive sorbent amounts (50 mg each) negatively impacted recovery. The established analytical method was validated for sensitivity and precision, with the LOQ of 0.01 mg/kg, facilitating trace residue analysis in rice samples following unmanned aerial pesticide application. While adjuvant use increased initial attachment, residues remained below MRL at harvest, ensuring safety. This research underscores the importance of optimizing extraction and purification methods for accurate residue analysis, offering valuable insights for improving agricultural chemical management and food safety.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14135607/s1, Figure S1: Product ion mass spectra of tricyclazole ($[M+H]^+$) at different collision energies (CEs). (a) CE at −20 V and (b) CE at −40 V; Figure S2: Optimization of collision energy (CE) for tricyclazole product ions. (a) product ion at m/z 163.1, (b) product ion at m/z 136.1, (c) product ion at m/z 109.1, (d) product ion at m/z 65.1; Figure S3: Calibration curve for tricyclazole matrix-matched standard solution; Table S1: Optimized multiple reaction monitoring (MRM) parameters and retention time (t_R) for tricyclazole analysis by LC-MS/MS; Table S2: Recovery rates ($n = 3$) for three different methods with the addition of formic acid (FA) to the extraction solvent in brown rice. Each method was conducted without purification steps; Table S3: Residual concentrations of tricyclazole in mature grain (brown rice) at 10 days after treatment (DAT) following aerial spraying with various adjuvants.

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References

1. Kunova, A.; Pizzatti, C.; Cortesi, P. Impact of tricyclazole and azoxystrobin on growth, sporulation and secondary infection of the rice blast fungus, Magnaporthe oryzae. *Pest Manag. Sci.* **2013**, *69*, 278–284. [CrossRef] [PubMed]
2. Wheeler, M.H. Melanin biosynthesis inVerticillium dahliae: Dehydration and reduction reactions in cell-free homogenates. *Exp. Mycol.* **1982**, *6*, 171–179. [CrossRef]
3. Thieron, M.; Pontzen, R.; Kurahashi, Y. Carpropamid: A rice fungicide with two modes of action. *Pflanzenschutz-Nachrichten Bayer* **1998**, *51*, 257–278.
4. Muthayya, S.; Sugimoto, J.D.; Montgomery, S.; Maberly, G.F. An overview of global rice production, supply, trade, and consumption. *Ann. N. Y. Acad. Sci.* **2014**, *1324*, 7–14. [CrossRef]
5. Ali, S.; Ullah, M.I.; Sajjad, A.; Shakeel, Q.; Hussain, A. Environmental and Health Effects of Pesticide Residues. In *Sustainable Agriculture Reviews 48: Pesticide Occurrence, Analysis and Remediation Volume 2 Analysis*; Inamuddin, Ahamed, M.I., Lichtfouse, E., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 311–336.
6. Rani, L.; Thapa, K.; Kanojia, N.; Sharma, N.; Singh, S.; Grewal, A.S.; Srivastav, A.L.; Kaushal, J. An extensive review on the consequences of chemical pesticides on human health and environment. *J. Clean Prod.* **2021**, *283*, 124657. [CrossRef]
7. Zhao, R.; Sun, Z.; Bird, N.; Gu, Y.-C.; Xu, Y.; Zhang, Z.-H.; Wu, X.-M. Effects of tank-mix adjuvants on physicochemical properties and dosage delivery at low dilution ratios for unmanned aerial vehicle application in paddy fields. *Pest Manag. Sci.* **2022**, *78*, 1582–1593. [CrossRef]
8. Kim, C.J.; Yuan, X.; Kim, M.; Kyung, K.S.; Noh, H.H. Monitoring and risk analysis of residual pesticides drifted by unmanned aerial spraying. *Sci. Rep.* **2023**, *13*, 10834. [CrossRef]
9. Carreño Ruiz, M.; Bloise, N.; Guglieri, G.; D'Ambrosio, D. Numerical Analysis and Wind Tunnel Validation of Droplet Distribution in the Wake of an Unmanned Aerial Spraying System in Forward Flight. *Drones* **2022**, *6*, 329. [CrossRef]
10. Løfstrøm, P.; Bruus, M.; Andersen, H.V.; Kjær, C.; Nuyttens, D.; Astrup, P. The OML-SprayDrift model for predicting pesticide drift and deposition from ground boom sprayers. *J. Pestic. Sci.* **2013**, *38*, 129–138. [CrossRef]
11. Khanal, D.; Dhital, A.; Neupane, A.; Paudel, K.; Shrestha, M.; Upadhyaya, N.; Bhandari, R.; Pandey, P. Insecticide residue analysis on vegetable crops through Rapid Bioassay of Pesticide Residue (RBPR) technique in Nepal. *J. King Saud Univ. Sci.* **2023**, *35*, 102671. [CrossRef]
12. Liu, L.; Yuki, H.; Qin, Y.; Zhou, H.; Lin, J. Rapid Analysis of Multiresidual Pesticides in Agricultural Products by Gas Chromatography-mass Spectrometry. *Chin. J. Anal. Chem.* **2006**, *34*, 783–786. [CrossRef]
13. Liu, L.-B.; Hashi, Y.; Qin, Y.-P.; Zhou, H.-X.; Lin, J.-M. Development of automated online gel permeation chromatography–gas chromatograph mass spectrometry for measuring multiresidual pesticides in agricultural products. *J. Chromatogr. B* **2007**, *845*, 61–68. [CrossRef]
14. Lee, S.J.; Park, H.J.; Kim, W.; Jin, J.S.; Abd El-Aty, A.M.; Shim, J.-H.; Shin, S.C. Multiresidue analysis of 47 pesticides in cooked wheat flour and polished rice by liquid chromatography with tandem mass spectrometry. *Biomed. Chromatogr.* **2009**, *23*, 434–442. [CrossRef]
15. Pareja, L.; Fernández-Alba, A.R.; Cesio, V.; Heinzen, H. Analytical methods for pesticide residues in rice. *TrAC Trends Anal. Chem.* **2011**, *30*, 270–291. [CrossRef]
16. Lee, J.; Kim, L.; Shin, Y.; Lee, J.; Lee, J.; Kim, E.; Moon, J.-K.; Kim, J.-H. Rapid and simultaneous analysis of 360 pesticides in brown rice, spinach, orange, and potato using microbore GC-MS/MS. *J. Agric. Food Chem.* **2017**, *65*, 3387–3395. [CrossRef]
17. Lee, J.; Shin, Y.; Lee, J.; Lee, J.; Kim, B.J.; Kim, J.-H. Simultaneous analysis of 310 pesticide multiresidues using UHPLC-MS/MS in brown rice, orange, and spinach. *Chemosphere* **2018**, *207*, 519–526. [CrossRef] [PubMed]
18. Mardani, Z.; Shakoori, A.; Peiravian, F.; Nouri, L.; Salamzadeh, J. Development of A Liquid Chromatography-Mass Spectrometry Technique for Evaluation of Multi-class Pesticides in Rice Samples. *Iran. J. Pharm. Res.* **2021**, *20*, 165–174.
19. Kim, S.-H.; Lee, Y.-H.; Jeong, M.-J.; Gwon, D.-Y.; Lee, J.-H.; Shin, Y.; Choi, H. LC-MS/MS Method Minimizing Matrix Effect for the Analysis of Bifenthrin and Butachlor in Chinese Chives and Its Application for Residual Study. *Foods* **2023**, *12*, 1683. [CrossRef] [PubMed]
20. EURL. European Union Reference Laboratories for Residues of Pesticides: Datapool. Available online: https://www.eurl-pesticides-datapool.eu/ (accessed on 7 August 2023).
21. Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431. [CrossRef]
22. Yang, S.-H.; Shin, Y.; Choi, H. Simultaneous analytical method for 296 pesticide multiresidues in root and rhizome based herbal medicines with GC-MS/MS. *PLoS ONE* **2023**, *18*, e0288198. [CrossRef]
23. Jeong, M.-J.; Kim, S.-H.; Eun, H.-R.; Lee, Y.-J.; Kim, S.-M.; Baek, J.-W.; Lee, Y.-H.; Shin, Y. Column Comparison for the Separation of Ferimzone Z and E Stereoisomers and Development of Trace Residue Analysis Method in Brown Rice Using HPLC-MS/MS. *Kor. J. Environ. Agric.* **2023**, *42*, 203–210. [CrossRef]

24. Shin, Y.; Lee, J.; Lee, J.; Lee, J.; Kim, E.; Liu, K.-H.; Lee, H.S.; Kim, J.-H. Validation of a multiresidue analysis method for 379 pesticides in human serum using liquid chromatography–tandem mass spectrometry. *J. Agric. Food Chem.* **2018**, *66*, 3550–3560. [CrossRef]
25. Shin, Y.; Lee, J.; Park, E.; Lee, J.; Lee, H.S.; Kim, J.-H. A Quantitative tandem mass spectrometry and scaled-down QuEChERS approach for simultaneous analysis of pesticide multiresidues in human urine. *Molecules* **2019**, *24*, 1330. [CrossRef] [PubMed]
26. Lehotay, S.J.; Maštovská, K.; Yun, S.J. Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *J. AOAC Int.* **2005**, *88*, 630–638. [CrossRef]
27. Koesukwiwat, U.; Lehotay, S.J.; Mastovska, K.; Dorweiler, K.J.; Leepipatpiboon, N. Extension of the QuEChERS method for pesticide residues in cereals to flaxseeds, peanuts, and doughs. *J. Agric. Food Chem.* **2010**, *58*, 5950–5958. [CrossRef] [PubMed]
28. Brosnan, B.; Coffey, A.; Arendt, E.K.; Furey, A. The QuEChERS approach in a novel application for the identification of antifungal compounds produced by lactic acid bacteria cultures. *Talanta* **2014**, *129*, 364–373. [CrossRef] [PubMed]
29. Ferrer, C.; Lozano, A.; Agüera, A.; Girón, A.J.; Fernández-Alba, A.R. Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. *J. Chromatogr. A* **2011**, *1218*, 7634–7639. [CrossRef]
30. Ellis, M.C.B.; Tuck, C.R.; Miller, P.C.H. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. *Crop Prot.* **1997**, *16*, 41–50. [CrossRef]
31. De Schampheleire, M.; Nuyttens, D.; Baetens, K.; Cornelis, W.; Gabriels, D.; Spanoghe, P. Effects on pesticide spray drift of the physicochemical properties of the spray liquid. *Precis. Agric.* **2009**, *10*, 409–420. [CrossRef]