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Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS) for High-Throughput Screening of Pesticides in Rice Samples Collected in Bangladesh and Saudi Arabia

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Abstract: Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS) with modified QuEChERS sample preparation has been applied to the high-throughput screening of pesticide residuals in rice collected from Bangladesh and Saudi Arabia markets. Both countries consume high volumes of rice, which is a fundamental food for their populations. We report optimized sample preparation and mass spectrometry analysis protocols, which can be rapidly deployed in analytical laboratories. The screening of four groups (organophosphorus, synthetic pyrethroid, organonitrogen, and organochlorine) of a total of 115 pesticides can be performed within ~10 min using a matrix-matched calibration. For most compounds, the limits of detection and quantification (LOD/LOQ) are well below the maximum residue levels (MRLs) of the main regulators. The method generally demonstrates acceptable recovery values (91 compounds 75–125% and 10 compounds 30–75%). Out of 55 rice samples analyzed, 16 samples (29%) contained pesticide residues above LOQ. Four samples contained chlorpyrifos with concentrations ranging from 21.3 to 71.9 μg/kg, ten samples contained tebuconazole (34.7–69.0 μg/kg), and three samples contained pirimiphos methyl (10.7–20.7 μg/kg). The concentrations of the pesticide residues detected in these samples are well below MRL of FAO/WHO (chlorpyrifos, 500 μg/kg; tebuconazole, 1500 μg/kg; pirimiphos methyl, 7000 μg/kg).

Keywords: mass spectrometry; food contaminants detection; GC-MS/MS; pesticides analysis in food; analytical methods

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1. Introduction

The screening of food products for pesticides is an important and rapidly evolving field. Some standard analytical procedures involving LC-MS [1–8] and GC-MS [9–16] have been developed and published by organizations, such as ISO [17]. These standards typically provide general guidance for analysis, including recommendations for using tandem mass spectrometry over single quadrupole mass analysis in high matrix samples, as well as guidelines for sample preparation, evaluation, and presentation of analytical results. However, the specific requirements for analytical methods can vary widely from country to country. In some developing countries, these requirements are often unregulated, creating challenges for food laboratories. Both LC-MS and GC-MS, which are the main analytical techniques for analysis of the pesticide residuals in food, have many advantages over other methods. These are a superior detection limit down to a lower *ppb-* range and a high selectivity, which is achieved via the combination of chromatography (separation of the molecules based on their chemical properties) and mass spectrometry detection.

Tandem mass spectrometry (MS/MS) is also usually equipped with two quadrupole mass filters, separated by a nitrogen-filled cell at lower pressures. The molecules of interest are first isolated in the first quadrupole, accelerated towards the fragmentation cell, and then the fragments (called quantitation ions), in turn, are selected by the second quadrupole and detected. This provides the technique with the additional layer of selectivity necessary for the unambiguous separation of the analyte molecules from abundant matrix ions.

In this work, we focus on the analysis of rice, which is a fundamental food in Saudi Arabia and is regularly consumed by a substantial proportion of the population [18,19]. Several studies have reported on the analysis of toxic trace elements, notably arsenic, in rice in Saudi Arabia [19–21], with concentrations as high as 464 μg/kg [21]. Among limited research on pesticides in Saudi Arabia, a study conducted by [19] revealed that among the 294 pesticides examined, 15 were present in rice. Out of these, six were fungicides and nine were insecticides, with detection frequencies of 22% and 26%, respectively. The pesticide levels varied between 11 and 517 μg/kg, with the highest detection rate observed for carbendazim (13.5%) and the lowest rate for biphenyl (0.6%).

There are even few large-scale studies on pesticides relating to Bangladeshi rice, which is striking. Bangladesh is predominantly an agrarian country, for which the agriculture sector contributes about ~11% to the country's GDP [22]. Rice cultivation accounts for ~70% of the total agricultural production [23]. The total amount of pesticide used in Bangladesh for agricultural purposes was around 40,000 MT in 2021 [22].

This paper aims to simplify the analytical process for food analysts who wish to quickly implement high-throughput screening procedures for pesticides using GC-MS/MS equipment. We present a straightforward sample preparation procedure, describe the GC-MS experimental conditions, and outline a typical laboratory approach for method development and data evaluation. This is demonstrated through the screening of a large number of rice samples collected by our colleagues in Bangladesh and Saudi Arabia.

2. Materials and Methods

2.1. Samples Origin

Tangail is a district of the central region of Bangladesh. It is the largest district of the Dhaka division. The district encompasses 12 upazilas, 119 unions and 11 municipalities [24]. The southwest region of the district is occupied by the Jamuna floodplain and the southeast region contains the Brahmaputra floodplain. The soil in the Brahmaputra floodplain is generally well structured and looks dark gray in color. In the Jamuna floodplain, it is silty and sandy alluvium of gray loam [24]. The economy of Tangail is primarily based on agriculture, and rice (local and hybrid varieties) is the major crop growing in Tangail [24].

Twenty-five (n = 25) rice samples were collected from two upazilas (Delduar and Nagarpur) of the Tangail district (Appendix C contains the details of rice collected from Tangail). Rice samples were collected directly from farmers as well as local markets. Rice samples were oven-dried, ground to a powder and stored in a cool place for analysis.

2.2. Sample Preparation

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method uses a single-step buffered acetonitrile (MeCN) extraction and salting out the liquid–liquid partitioning from the water in the sample with $MgSO_4$ [25,26]. Dispersive-solid-phase extraction was conducted (clean-up was carried out to remove organic acids, excess water, and other components with a combination of primary secondary amine (PSA) sorbent and $MgSO_4$); then, the extracts were analyzed using mass spectrometry (MS) techniques after chromatographic analytical separation [9,13,27–29].

A slightly modified extraction process contained the following steps: a 7.5 g rice sample was weighed into a 50 mL extraction tube and 15 mL of HPLC-grade water containing 1% acetic acid was added to the rice and the sample was kept for soaking overnight. Acetonitrile (15 m) was then added to the tube, which was then shaken vigorously on a vortex mixer for 1 min at 2500 rpm. Anhydrous $MgSO_4$ (6 g) and anhydrous sodium acetate

(1.5 g) were added to the tube (extraction kit) and mixed again on a vortex mixer for 1 min at 2500 rpm and centrifuged at 3500 rpm for 10 min. Then, 10~15 mL supernatant extract (10–15 mL) was transferred to a 15 mL centrifuge tube and centrifuged at 5000 rpm for 10 min. Then, 1 mL of the supernatant liquid was transferred into a 2 mL microcentrifuge tube containing 150 mg $MgSO_4$ and 50 mg PSA (clean-up kit). Samples were vortexed for 1 min at 2500 rpm and centrifuged at 10,000 rpm for 5 min. The supernatant (0.5 mL) was then collected and transferred to a 2 mL autosampler vial for instrumental analysis. A controlled blank extract and matrix-matched calibration standards were also prepared by following the above protocol [25,27,30].

2.3. Gas Chromatography Mass Spectrometry: Experimental Conditions

Agilent tandem mass spectrometry (Santa Clara, CA, USA) equipment with the following modules was used: Agilent 7890B GC, Agilent 7010B GC MS Triple Quad, and the PAL RTC 120 autosampler.

For the chromatography column, the parameters were as follows: HP-5MS, 30 m, a 0.250 mm diameter, and a film of 0.25μ m. The oven parameters: initial 150 C, hold for 0 min., heating to 280 C @ 30 C/min, hold at 280 °C for 10 min. Post run: 10 min @ 120 °C. Splitless inlet was heated to 250 C and purged with He at 3 mL/min. The column flow was 1.2 mL/min. Sample injection was 2 μ L. Collision cell: the quench gas was He 2.25 mL/min and collision followed N_2 @ 1.5. mL/min. The quadrupole and transfer line were heated to 200 °C. The chromatography parameters (temperature steps, flow rate, injection volume, etc.) were optimized to deliver the best separation of the compounds within a reasonable time duration.

2.4. Method Development

The standard approach for developing and implementing new analytical methods in the laboratory involves using standards with known concentrations. For this work, four sets of pesticide standards were purchased from (Thames Restek UK Limited, High Wycombe, United Kingdom): organophosphorus compounds (set 32563 and set 32570), synthetic pyrethroid compounds (set 32568), organonitrogen compounds (set 32567), and organochlorine compounds (set 32564). The standards were originally bought as 100 μg/mL and then diluted in ethyl acetate using precision electronic pipettes (Sartorius, Göttingen, Germany) to an initial concentration of 200 μg/kg.

These solutions were utilized for method development, which involved multiple runs under varying experimental conditions (e.g., He flow, oven temperature program, and injection volume). The goal was to establish optimal experimental conditions to achieve maximum peak resolution, signal-to-noise ratio, and peak shape, as well as to determine the retention time for each pesticide. Mixing components from different sets was avoided to prevent potential chemical interactions and ion suppression/enhancement.

Additionally, the MS dwell time (the time the MS spends collecting each ion of interest) was limited by the number of ions of interest and their retention times, which can be very close for some pesticides. In this method, we analyzed 115 pesticides in 5 analytical standards across 5 independent analytical runs (i.e., each sample was analyzed 5 times). The dwell time in this case ranges from 10 to 100 ms, long enough to avoid compromising sensitivity. A very convenient feature of the vendor software is the "dynamic dwell time" adjustment, where the software calculates and adjusts the dwell time automatically based on retention time, and the total count rate is recalculated online automatically.

Typical total ion gas chromatograms (TICs) of the rice matrix spiked with 100 μg/kg of different pesticides standard mix (five sets) are presented in Figure 1. The chromatography peaks, which are the sum of all ions generated by the mass spectrometer at a certain elution time, exhibit reasonable shapes and heights. As expected, the ionization efficiencies of the pesticide components vary significantly, sometimes by several orders of magnitude. These ionization probabilities ultimately define the method's sensitivity. While some pesticides (up to two at a time) may co-elute, this generally does not pose a problem because quantification is based on MS/MS transitions.

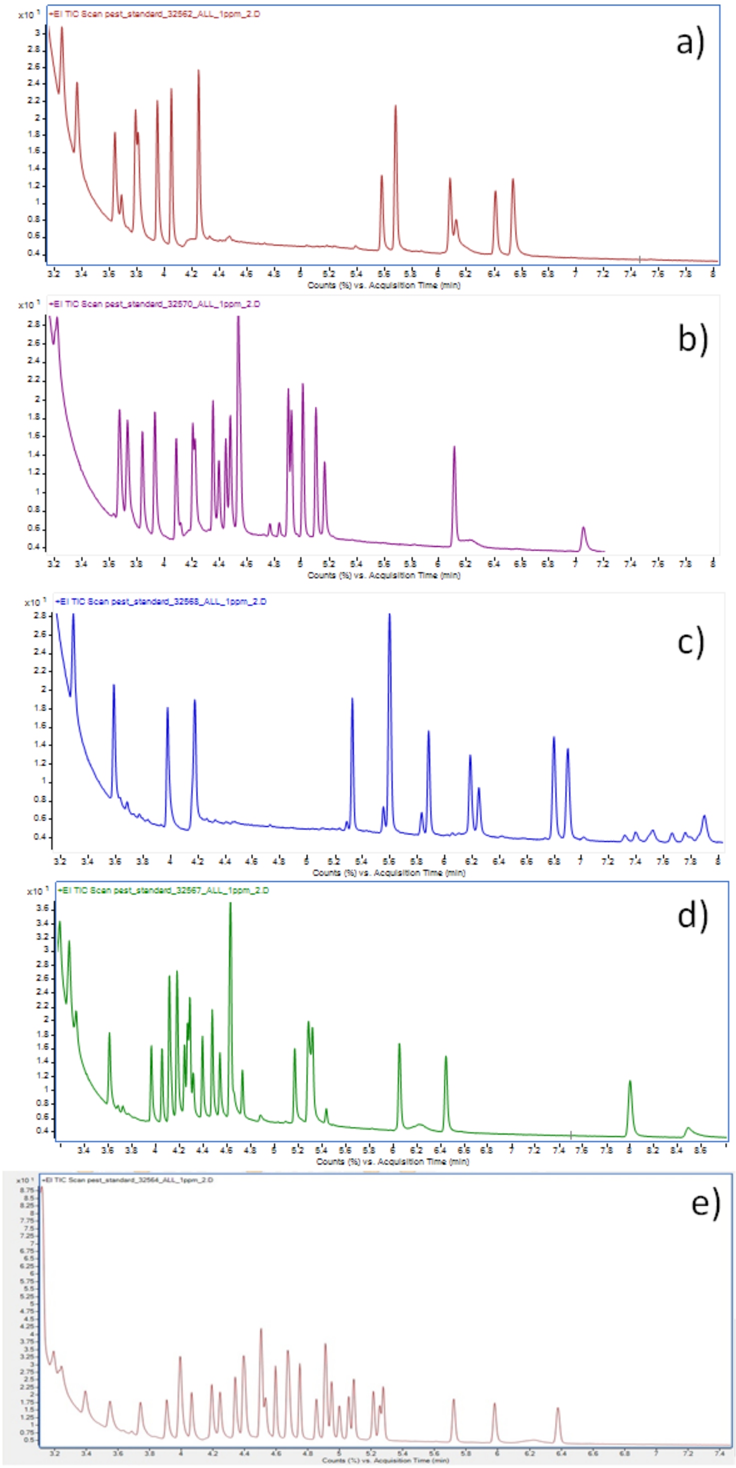

Figure 1. Typical total ion gas chromatograms (TICs) of the rice matrix spiked with 100 µg/kg of different pesticides standard mix (**a**) organophosphorus compounds (set 32563), (**b**) organophosphorus compounds (set 32570), (**c**) synthetic pyrethroid compounds (set 32568), (**d**) organonitrogen compounds (set 32567), and (**e**) organochlorine compounds (set 32564).

Appendix A summarizes the pesticides analyzed using this method, their retention times, as well as the ion transitions and associated collision energies and ion ratios. The identification is based on the two ion transitions (quantifier and qualifier). The well-known experimental approach is to isolate the parent ion on the first quadrupole filter, fragment it using a nitrogen-filled gas cell (the total pressure is 10^{-5} mBar), and then detect the fragments on the second quadrupole working in the scanning mode. Two transitions from each pesticide are measured and the ratio of the quantifier and qualifier ions are determined off-line and serves for the data quality control—should the matrix ions interfere with the pesticide signal, this ratio will change unpredictably from the theoretical one determined using the calibration standards.

2.5. Calibration Procedure and Long Stability Tests

In Figure 2, the screenshots derived from the data analysis software are presented for the case of chlorpyrifos for which the quantifier (314 → 258 a.m.u.) and qualifier (314 → 286 a.m.u.) transitions were used. From the experiments, it was established that the rice matrix generally enhances the ionization efficiencies of the pesticides in the ion source. The effect is not as dramatic as can be observed when using LC-MS equipment for which up to several orders of magnitude variations in ionization signal can be registered but can contribute up to ~30% to GC-MS/MS method uncertainties.

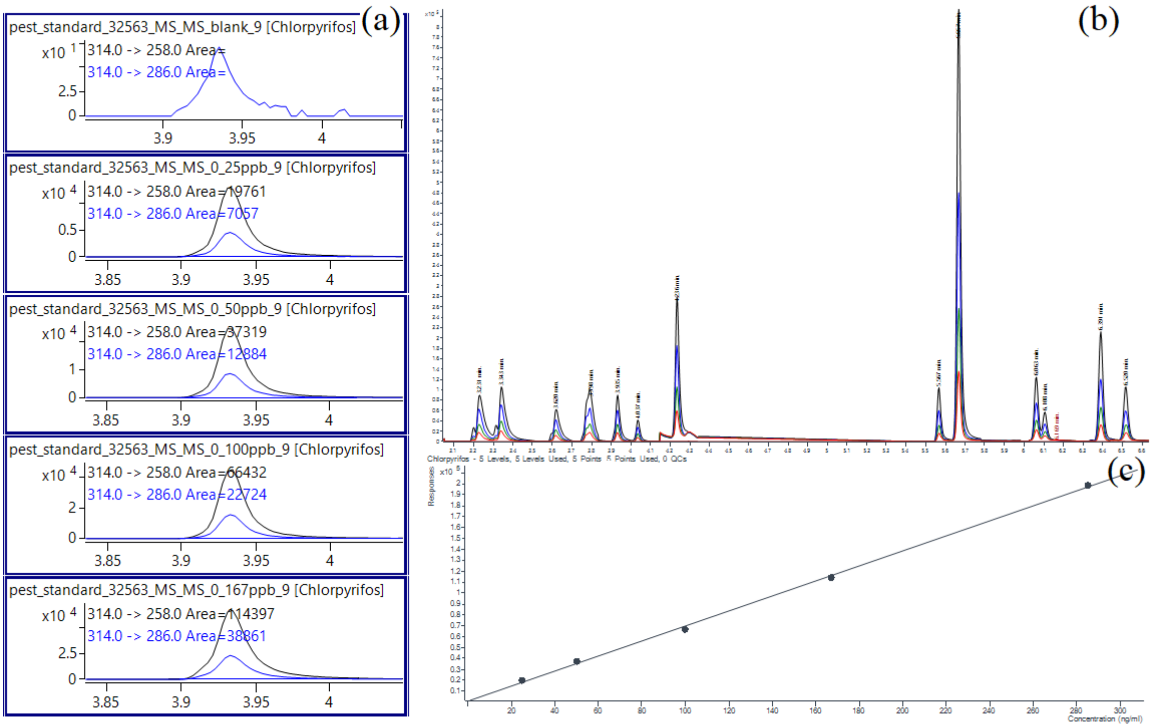

Figure 2. Screenshots from the data analysis software, the examples of (**a**) the GCMS signal from the matrix-matched chlorpyrifos calibration standards for the blank, 25, 50, 100, and 160 µg/kg with the quantifier (314 → 258 a.m.u.)/qualifier (314 → 286 a.m.u.) transitions, (**b**) the overlaid GC signals for these calibrants with other pesticides components also visible, and (**c**) the resultant linear calibration curve for this concentrations range.

During analysis, we used matrix-matched calibration. Several grams of rice were used to prepare the blank rice matrix, which was later spiked with pesticides to generate a set of calibration standards with concentrations of 0 (blank), 25, 100, and 160 µg/kg. Prior to spiking, this matrix was thoroughly investigated for possible contamination with pesticides and was found to be clean (no ion signal was detected for any of the components). The detector, as expected, is linear within this concentration range. Concentrations of up to 1 mg/kg can be, in principle, accurately determined using these linear calibration curves.

In Figure 3, the results of the long stability tests are presented. The pure rice matrix was spiked with ~45 µg/kg of Fenchlophos (Ronnel) and the sample was analyzed 50 times. The total analysis time includes measuring the calibration curves. The oven heating–cooking circle took ~20 h to complete. The retention time of Fenchlophos was stable to within several seconds, and qualifier/quantifier ion ratios were stable to <1% (a standard deviation of the detected ratios); both are expected for this type of equipment. The average for measured concentrations, without recalibration, was 43 µg/kg with a standard deviation of ~9%, which constitutes the instrumental drift during 20 h measurements. This drift can be addressed by recalibrating, e.g., every 7 h (or every ~15 samples). In this case, from the experiments, the standard deviation on concentration measurements is ~4%, which is comparable to the expected precision for this equipment.

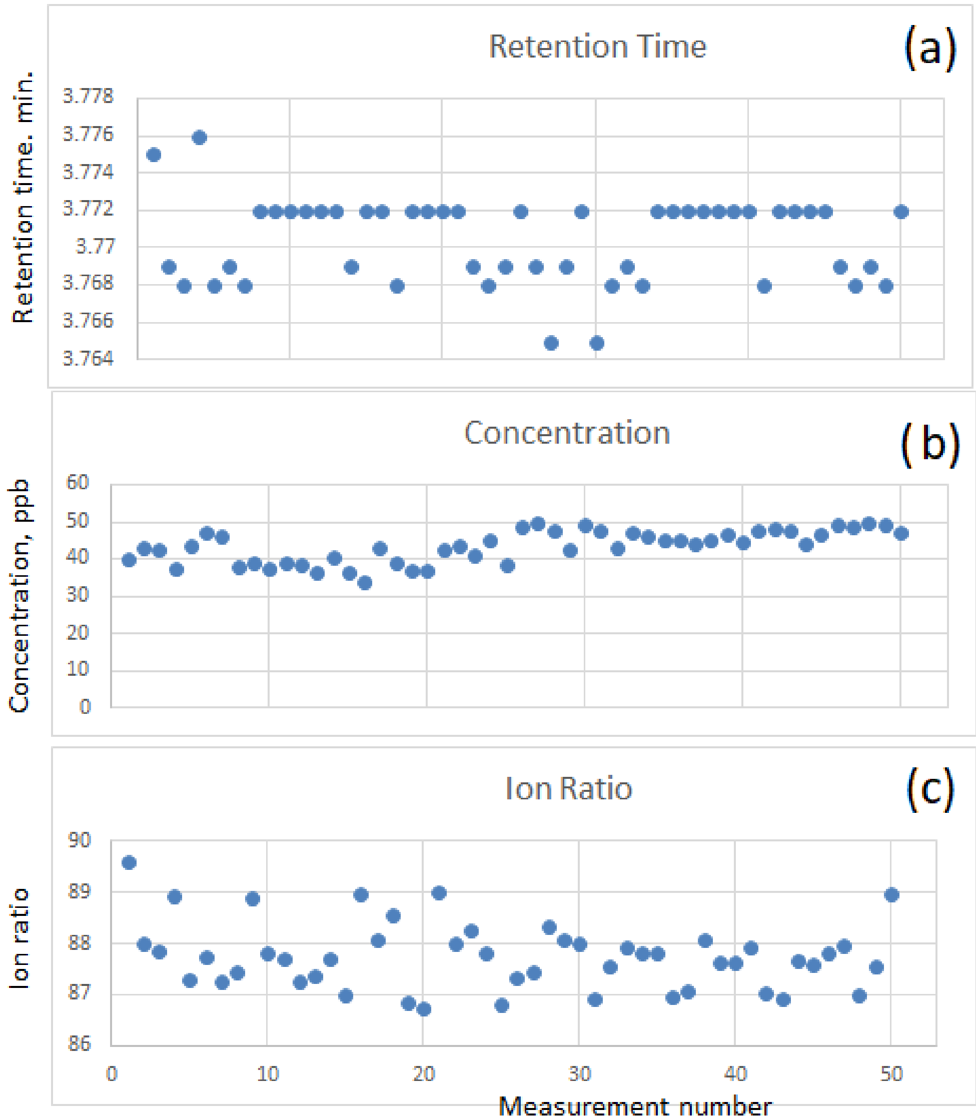

Figure 3. A long stability test during which 50 rice samples spiked with ~45 µg/kg of fenchlorphos (Ronnel) was measured within ~20 h. Experimental values for reproducibility of (**a**) retention time (min.), (**b**) concentration measurements (µg/kg), and (**c**) the ratios of quantifier and qualifier ions.

3. Results

3.1. Estimation of Limits of Detection (LOD) and Quantification (LOQ)

Limits of detection and quantification are important method parameters in terms of indicating the method's ability to detect the compounds (LOD, especially important in high-throughput analysis) and quantify them with an acceptable level of uncertainty

(LOQ). These parameters, according to the International Committee on Harmonizatio, can be estimated from the calibration curves [31].

The typical measurements involved the construction of the calibration curves with the matrix-matched calibration samples having concentrations in the range of LOQ (µg/kg–range in our case) and performing a linear regression analysis. In this case, the standard error of the calibration curve (or standard deviation of the y-intercept) and the slope of the curve can be used for the calculation of the LOD and LOQ using the following formulas:

$$\text{LOD} = 3.3 \times (\text{STDEV of Intercept})/\text{slope; and LOQ} = 10 \times (\text{STDEV of intercept})/\text{slope}.$$

The calculated LODs and LOQs for the matrix-matched rice pesticide calibration samples are listed in Table 1 and also compared, where data available with the maximum residue levels adopted by FAO/WHO [32], EU [33], USA [34], Saudi Arabia [35], and Bangladesh [36].

Table 1. Limits of detection (LOD) and quantification (LOQ) calculated for the matrix-matched (rice) analytical standards for different groups of pesticides. Maximum residue levels (MRLs) adopted by FAO/WHO, EU, USA, Saudi Arabia, and Bangladesh are also indicated.

	Pesticides	**RT, min**	**LOD, µg/kg**	**LOQ, µg/kg**	**Maximum Residue Level (MRL), µg/kg**				
					FAO/WHO Codex	**EU**	**Saudi Arabia**	**USA**	**Bangladesh**
1	Azinphos ethyl	6.5	32.3	97.8		50			
2	Chlorpyrifos	3.9	3.2	9.8	500	500	500	100	500
3	Chlorpyrifos methyl	3.6	7.4	22.6		3000	100	6000	
4	Diazinon	3.2	4	12.1		10			
5	EPN	5.6	28.8	87.3					
6	Fenitrothion	3.7	10.6	32.4	6000	50		20	
7	Isazophos	3.3	14.6	44.4					
8	Phosalone	6	36.6	111.0		10			
9	Phosmet	5.6	50.5	153		10			
10	Pirimiphos ethyl	4	8.7	26.6					
11	Pirimiphos methyl	3.7	4.3	13.1	7000	500	7000		500
12	Pyraclofos	6.5	49.8	151.1					
13	Pyrazophos	6.3	33.7	102.3		10			
14	Pyridaphenthion	5.5	32.2	97.6					
15	Quinalphos	4.2	55.4	167.9		10			10
16	Acrinathrin	6.2	12.4	37.6					
17	Allethrin	4.1	51.6	156.5					
18	Anthraquinone	3.9	2.5	7.7					
19	Bifenthrin	5.5	1.1	3.3		10			50
20	cis- Permethrin	6.7	2.3	7.2	2000	50		50	
21	Cypermethrin	6.1	9.9	30.1	2000	2000	2000		2000
22	Deltamethrin	9.9	22.3	67.7	2000	1000	2000	1500	
23	Fenvalerate	8.8	15.9	48.3		20	2000	2500	
24	Flucythrinate	8	13.2	40					
25	lambda Cyhalothrin	6.1	9	27.4	1000	200	1000	1000	
26	Phenothrin	5.8	11	33.5		50		10	
27	Resmethrin	4.2	158.3	479.8		20		3000	
28	tau- Fluvalinate	9.1	21.6	65.7		10			
29	Tefluthrin	3.2	18.5	56		10			
30	Tetramethrin	5.5	14.2	43.2					
31	trans- Permethrin	6.8	4	12.3	2000	50	2000		
32	Transfluthrin	3.5	27.8	84.3					
33	2,4′ DDD	4.7	2.4	7.3					
34	2,4′ DDE	4.3	2.8	8.7		50			
35	2,4′ DDT	4.9	4.4	13.5	100		100	500	

Table 1. *Cont.*

	Pesticides	RT, min	LOD, µg/kg	LOQ, µg/kg	Maximum Residue Level (MRL), µg/kg				
					FAO/WHO Codex	EU	Saudi Arabia	USA	Bangladesh
36	2,4′ Methoxychlor	5.2	21.1	64.2					
37	4,4′ DDD	4.8	59.8	181.3					
38	4,4′ DDE	4.5	2.3	7.2		50			
39	4,4′ DDT	5.2	9.4	28.6	100		100	500	
40	4,4′ Dichlorobenzophenone	3.9	12.2	37.1					
41	Aldrin	3.9	23.6	71.6	20	10	20	20	
42	alpha- BHC	3.1	6.7	20.5		10			
43	beta- BHC	3.2	71.5	216.7		10			
44	Chlorbenside	4.3	2.8	8.7		10			
45	Chlorfenson (Ovex)	4.5	4.2	12.7		10			
46	cis- Chlordane	4.4	5.7	17.3	20		20		
47	cis- Nonachlor	4.9	6.2	18.8					
48	delta- BHC	3.2	6	18.2		10			
49	Dieldrin	4.4	5.7	17.5	20	10	20	20	
50	Endosulfan ether	3.5	3.8	11.6					
51	Endosulfan I	4.3	16.5	50.1					
52	Endosulfan II	4.8	13.6	41.3		50			
53	Endosulfan sulfate	5.2	12.7	38.7					
54	Endrin	4.8	1.4	4.3		10			
55	Endrin aldehyde	5	3.4	10.5					
56	Endrin ketone	5.7	3.1	9.6					
57	Ethylan (Perthane)	4.7	2.5	7.7					
58	Fenson	4.1	2.1	6.4					
59	Gamma BHC (Lindane)	3.4	8.6	26		10			
60	Heptachlor	3.7	36.8	111.7	20	10	20	30	
61	Heptachlor epoxide (isomer B)	4.2	32.3	98					
62	Isodrin	4.1	6.1	18.7					
63	Mirex	6.3	2.2	6.7					
64	Pentachlorothioanisole	3.9	3.8	11.6					
65	Tetradifon	5.9	1.8	5.7		10			
66	trans- Chlordane	4.3	2.2	6.6	20		20		
67	trans- Nonachlor	4.4	8	24.4					
68	Bupirimate	4.6	3.8	11.4		10			
69	Chlorfenapyr	4.7	6.1	18.5		20			
70	Cyprodinil	4.1	4.1	12.5				10	
71	Etofenprox	7.9	6.7	20.4	10	10	10	10	10
72	Fenarimol	6.4	2.3	6.9		20			
73	Fipronil	4.1	4.3	13.1	10	5	10	40	10
74	Fludioxonil	4.5	3.7	11.2	50	10	50	20	
75	Fluridone (Sonar)	8.5	28.3	86				100	
76	Flusilazole	4.6	1.5	4.5		10			10
77	Flutriafol	4.4	4.6	14		1000		500	
78	Folpet	4.2	19.1	58					
79	Hexazinone	5.2	11.6	35.2					
80	Lenacil	5.1	2.4	7.4		100			
81	MGK-264	4	5.2	15.9					
82	Myclobutanil	4.6	4.8	14.7		10		30	
83	Paclobutrazol	4.3	5.1	15.4		10			
84	Penconazole	4.1	6.4	19.5		10			
85	Procymidone	4.2	6.3	19.1					
86	Pyrimethanil	3.2	2.5	7.8		50			
87	Pyriproxyfen	6	3.6	10.9		50		1100	
88	Tebuconazole	5.3	5.3	16.1	1500	1500	1500		1500
89	Terbacil	3.3	46.2	140.2					
90	Terbuthylazine	3.1	1.8	5.6		10			

Table 1. *Cont.*

Pesticides		RT, min	LOD, μg/kg	LOQ, μg/kg	Maximum Residue Level (MRL), μg/kg				
					FAO/WHO Codex	EU	Saudi Arabia	USA	Bangladesh
91	Triadimefon	3.9	4.7	14.4		10			
92	Triadimenol	4.2	4.4	13.6		10			
93	Triflumizole	4.2	14.4	43.6		20			
94	Vinclozolin	3.5	1.5	4.6		10			
95	Bromfenvinfos methyl	4.2	10.6	32.2					
96	Bromphos ethyl	4.3	4.3	13.2		10			
97	Bromphos methyl	4	18	54.7					
98	Carbophenothion	5	5.4	16.4					
99	Chlorfenvinphos	4.1	5.1	15.4		10			
100	Chlorthiophos	4.9	3.1	9.3					
101	Coumaphos	7	16.3	49.4					
102	Edifenphos	5.1	14.5	44.1					20
103	Ethion	4.8	5.3	16		10			30
104	Fenamiphos	4.4	8.4	25.4					
105	Fenchlorphos (Ronnel)	3.7	8.3	25.2					
106	Fenthion	3.9	10	30.5	50	10	50		50
107	Iodofenphos	4.5	13.8	42					
108	Leptophos	6	6.4	19.4					
109	Malathion	3.8	7.8	23.6		8000		8000	
110	Profenofos	4.5	6.2	18.9		10			
111	Prothiofos	4.5	2.1	6.3					
112	Sulfotepp	3.2	223	676					
113	Sulprofos	4.9	4.4	13.4					
114	Tetrachlorvinphos	4.3	10.6	32.3					
115	Tolclofos methyl	3.6	7.9	14.2		10			

Based on the experimental data, the method demonstrates strong detection ability. For many compounds, the detection limits are well below the MRL of the main regulators, for example, the abundant and well-controlled chlorpyrifos (LOD = 3.2 μg/kg; LOQ = 9.8 μg/kg; MRL = 100–500 pbb), pirimiphos methyl (LOD = 4.3 μg/kg; LOQ = 13.1 μg/kg; MRL = 500–7000 μg/kg), and etebuconazole (LOD = 5.3 μg/kg; LOQ = 16.1 μg/kg; MRL = 1500). Similarly, with cis-permethrin, cypermethrin, deltamethrin, malathion, and others, the LOD and LOQ are several orders of magnitude lower than their corresponding MRLs of 2000–8000 μg/kg.

A large group of compounds have LOD/LOQ levels comparable with the regulators (usually at the level of 10–20 μg/kg), e.g., diazinon phenothrin, most DDDs, DDTs, DDEs, tetradifon, most organonitrogen compounds and most organophosphorus compounds.

Among those with high LOD/LOQ are some synthetic pyrethroid compounds: (resmethrin, and deltamethrin, both having high MRLs), phosalone, pyraclofos, pyrazophos, resmethrin, sulfotepp. These compounds have either low ionization efficiencies or weakly interact with the chromatography column, and this elute at the beginning of the analysis partially overlaps with the solvent tail. Chromatography, in principle, can be improved by reducing the temperature gradient and decreasing the helium flow rate. However, this will considerably increase the analysis time, which is not advisable for the high-throughput analysis we are pursuing.

3.2. Recoveries of Pesticides after Sample Preparation

The method recoveries mostly characterize the ability of the sample preparation procedure to extract the analyte from the sample matrix. In this experiment, the rice powder was spiked with the pesticide standards before sample preparation. After drying, the powder was thoroughly mixed and then the samples were prepared according to the sample procedure described earlier. The resultant concentrations of the pesticides in the vials for analysis were 50 μg/kg. Five usual calibration standard sets were used. A total of 15 spiked samples were prepared (5 standard sets with three samples of each standard set) and measured independently. The blank samples were also prepared and analyzed to make sure the rice matrix did not contain any detectable levels of pesticides, intrinsic or from possible cross-contamination.

The experimental recoveries are presented in Table 2. The standard deviation on recoveries (n = 3) is also presented. The method generally demonstrates acceptable recovery values. In total, 58 pesticides have recoveries of 75–100%, and 33 pesticides have recoveries of 100–125%. The lower part of the spectrum is 10 pesticides with recoveries of 30–75% and 11 pesticides with recoveries above 125%. The standard deviation of recoveries is on average 11% and varied usually 2–26%, with the lowest found for chlorpyrifos (~1%).

Table 2. Typical recoveries (%) and standard deviation on recovery measurements (n = 3) of the pesticides in rice samples after sample preparation.

Name	Recovery, %	St. Deviation, n = 3
2,4′ DDD	85	2
2,4′ DDE	84	4
2,4′ DDT	89	4
2,4′ Methoxychlor	85	4
4,4′ DDD	81	4
4,4′ DDE	75	3
4,4′ DDT	88	5
4,4′ Dichlorobenzophenone	79	3
Acrinathrin	112	13
Aldrin	76	2
Allethrin	53	13
alpha BHC	96	15
Anthraquinone	82	7
Azinphos ethyl	107	21
beta BHC	63	32
Bifenthrin	89	2
Bromfenvinfos methyl	93	23
Bromphos ethyl	102	28
Bromphos methyl	87	8
Bupirimate	105	6
Carbophenothion	91	6
Chlorbenside	76	11
Chlorfenapyr	104	5
Chlorfenson (Ovex)	95	5
Chlorfenvinphos	95	13
Chlorpyrifos methyl	107	23
Chlorpyrifos	109	1
Chlorpyrifos methyl	88	7
Chlorthiophos	89	4
cis Nonachlor	67	4
cis Permethrin	86	4
Coumaphos	111	15
Cypermethrin	122	12
Cyprodinil	102	5
delta BHC	75	3
Deltamethrin	116	13
Diazinon	85	13
Dieldrin	87	8
Edifenphos	96	24
Endosulfan ether	98	2
Endosulfan I	109	16
Endosulfan II	76	7
Endosulfan sulfate	100	9
Endrin	88	4
Endrin aldehyde	45	5

Table 2. *Cont.*

Name	Recovery, %	St. Deviation, n = 3
Endrin ketone	91	5
EPN	93	5
Ethion	97	5
Ethylan (Perthane)	88	2
Etofenprox	104	5
Fenarimol	114	9
Fenchlorphos (Ronnel)	80	4
Fenitrothion	78	10
Fenson	69	5
Fenthion	81	6
Fenvalerate	112	9
Fipronil	109	4
Flucythrinate	115	13
Fludioxonil	110	11
Flusilazole	116	13
Flutriafol	112	6
Folpet	12	2
gamma BHC (Lindane)	144	85
Heptachlor	75	17
Heptachlor epoxide (isomer B)	38	10
Hexazinone	127	22
Iodofenphos	99	6
Iprodione	102	5
Isazophos	97	18
Isodrin	77	20
lambda Cyhalothrin	118	9
Lenacil	128	29
Leptophos	90	7
Malathion	92	12
MGK-264	73	4
Mirex	53	5
Myclobutanil	115	10
Paclobutrazol	116	4
Penconazole	107	13
Pentachlorothioanisole	33	18
Phenothrin	82	4
Phosalon	132	28
Phosalone	130	26
Phosmate	159	36
Phosmet	162	34
Pirimiphos ethyl	76	3
Pirimiphos methyl	83	18
Procymidone	110	5
Profenofos	90	16
Prothiofos	82	3
Pyrazophos	107	14
Pyridaphenthion	107	14
Pyrimethanil	96	14
Pyriproxyfen	114	6
Quinalphos	77	9
Sulfotepp	96	19
Sulprofos	79	4
tau Fluvalinate	126	26
Tebuconazole	134	13
Tefluthrin	92	4
Terbacil	163	99
Terbuthylazine	96	49

Table 2. *Cont.*

Name	Recovery, %	St. Deviation, n = 3
Tetrachlorvinphos	90	25
Tetradifon	94	3
Tetramethrin	163	25
Tolclofos methyl	74	5
trans Chlordane	84	6
trans nonachlor	90	11
trans Permethrin	88	5
Transfluthrin	88	18
Triadimefon	102	8
Triadimenol	113	10
Triflumizole	110	18
Vinclozolin	106	4

3.3. Analysis of the Rice Samples Collected in Bangladesh and Saudi Arabia

We have analyzed 55 rice samples (samples number 2–31 were collected in Saudi Arabia and 32–56 in Bangladesh) (Table 3). Sixteen samples contained pesticide residues above LOQ levels (29% of the total). Three pesticide compounds were detected. Four samples contained chlorpyrifos with concentrations ranging from 21.3 to 71.9 µg/kg. Ten samples contained tebuconazole (34.7–69.0 µg/kg), and three samples contained pirimiphos methyl (10.7–20.7 µg/kg). The concentrations of the pesticide residues detected in these samples are well below the MRL of main regulators (Table 4: chlorpyrifos, 500 µg/kg; tebuconazole, 1500 µg/kg; and pirimiphos methyl, 7000 µg/kg).

Table 3. Pesticide residuals were detected in the 55 samples collected in Bangladesh and Saudi Arabia.

Sample Number	Exp. Run 1, µg/kg	Exp. Run 2, µg/kg	Exp. Run 3, µg/kg	Mean, µg/kg	St. Dev
Chlorpyrifos					
3	28.6	18.8	16.4	21.3	6.5
15	29.8	26.8	24.6	27.1	2.6
20	75.4	70.6	69.8	71.9	3.0
56	23.4	23.2	21.2	22.6	1.2
Tebuconazole					
2	66.2	63.8	68.0	66.0	2.1
7	48.4	49.2	48.8	48.8	0.4
14	69.4	67.6	70.0	69.0	1.2
17	39	39.4	37.4	38.6	1.1
25	38.2	40	40.2	39.5	1.1
32	43.4	42.6	44.3	43.4	0.9
37	35	32.8	36.4	34.7	1.8
41	43.8	42.6	38.6	41.7	2.7
45	38.6	37.4	37	37.7	0.8
53	50.2	49.2	45.4	48.3	2.5
Pirimiphos methyl					
17	12.6	10.6	8.8	10.7	1.9
36	14.4	11.6	10.4	12.1	2.1
46	22.4	20.8	19	20.7	1.7

Table 4. The maximum residue levels (MRLs) (µg/kg) of chlorpyrifos, tebuconazole, and pirimiphos methyl adopted by FAO/WHO, EU, USA, Saudia Arabia, and Bangladesh [32–36].

Pesticide	MRL FAO/WHO Codex, µg/kg	MRL EU, µg/kg	MRL Saudi Arabia, µg/kg	MRL USA, µg/kg	MRL Bangladesh, µg/kg
Chlorpyrifos	500	500	500	100	500
Tebuconazole	1500	1500	1500		1500
Pirimiphos methyl	7000	500	7000		500

4. Discussion and Conclusions

While the pesticide residues found were all below the MRL limit, they were detected in a total of nine samples of Saudi rice (eight imported rice samples and one local rice sample) and eight samples of Bangladeshi rice. Regarding the Saudi Arabian rice samples, sample 20 had the highest residue concentration of the insecticide chlorpyrifos, with no significant difference from the study found by (Almutairi et al. 2021 [19]), and the lowest concentration was found in sample 3. The fungicide tebuconazole had the highest residue concentration in sample 14, while the lowest concentration was observed in sample 17; both of these concentrations were slightly higher than that of reported in (Almutairi et al. 2021 [19]) study. Pirimiphos-methyl was detected in only one sample: sample 17.

Multiple research works have been carried out to quantify several groups of pesticides in food items in Bangladesh, i.e., vegetables, fruits, fish and fish products, chicken meat, egg, milk, and dairy products [7,14,37–45]; however, not much has been carried out with a focus on rice or cereal grains and their by-products [46,47]. The mean concentrations of diazinon pesticide residue in rice grains collected from three fields of Gazipur district have been 560, 940, and 1680 µg/kg [46], In addition, fenitrothion has also been detected, with a mean concentration of 450 µg/kg [46]. Both pesticide residue concentrations in rice grains exceeded the regulatory values of the EU. Maize grain, flour and processed items (n = 90) collected from Dhaka have been analyzed for 27 pesticide residues using GC-MS. The mean concentrations of dichlorvos, methyl-parathion, chlorpyrifos, DDE, DDD, and DDT found in maize grains were 965, 44, 40, 7, 4, and 5 µg/kg [47]. The mean concentrations of dichlorvos, methyl-parathion, and chlorpyrifos in maize grain were higher than the MRL of the EU [33] but within the Bangladesh limit for chlorpyrifos in maize grains [36]. However, except for chlorpyrifos, all other detected pesticide residues were banned in Bangladesh for agricultural purposes [48].

Pesticides are frequently applied without precision in Bangladesh as well as other south and southeast Asian countries [49–53], which leads to several adverse effects on human health, from acute intoxication to chronic diseases that include various types of cancer (brain cancer, breast cancer, prostate cancer, bladder cancer, and colon cancer), Parkinson's disease, neurotoxicity, and diabetes [54–58]. The results of the study indicate that the pesticide residues analyzed and detected in rice samples from Bangladesh and Saudi Arabia, including chlorpyrifos, tebuconazole, and pirimiphos-methyl, were below the MRLs set by EU regulators. These results highlight the need for continuous monitoring and control measures to maintain food safety standards, which, in turn, protects public health in countries where rice is a staple food.

The GC-MS method demonstrated high efficacy, providing sensitivity in detecting and quantifying pesticides. The relatively low values of the limits of detection and limits of quantification indicate that the method has the potential to detect pesticides at low concentrations. Future applications and development of this method could further enhance the detection and analysis of a wide range of pesticide residues in different matrices. It could be used to monitor food safety and investigate other potential hazards, such as the presence of mycotoxins and heavy metal contamination, to improve public health outcomes.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Experimental retention times, quantifier and qualifier ion transitions and associated collision energies were used for quantitative analysis of pesticide residuals in rice.

				Quantifier Transition			**Qualifier Transition**			
	Compound Name	**RT, min**	**Dwell Time, ms**	**Ion1, a.m.u.**	**Ion 2, a.m.u.**	**CE1, eV**	**Ion1, a.m.u.**	**Ion2, a.m.u.**	**CE2, eV**	**Ion Ratio**
1	2,4′ DDD	4.7	18.0	235	199	15	235	165	20	31
2	2,4′ DDE	4.4	12.0	246	211	20	246	176	30	10
3	2,4′ DDT	5.0	14.6	235	199	20	235	165	20	26
4	2,4′ Methoxychlor	5.3	44.2	227	169	25	227	115	40	95
5	4,4′ DDD	4.9	15.2	235	199	15	235	165	20	25
6	4,4′ DDE	4.6	16.6	246	211	20	246	176	30	9
7	4,4′ DDT	5.2	26.1	235	199	20	235	165	20	7
8	4,4′ Dichlorobenzophenone	4.0	23.9	250	139	8	139	111	8	15
9	4,4′ Methoxychlor olefin	5.1	16.9	227	169	25	227	115	40	42
10	Acrinathrin	6.2	40.7	289	93	5	208	181	5	40
11	Aldrin	4.0	25.1	293	257	8	293	186	40	22
12	Allethrin	4.2	40.8	123	107	5	123	79	10	0.8
13	alpha BHC	3.2	57.6	219	183	5	219	145	25	29
14	Anthraquinone	4.0	65.9	208	180	10	208	152	22	90
15	Azinphos ethyl	6.5	40.7	160	132	5	160	105	12	50
16	Azinphos methyl	6.1	74.1	160	132	5	160	105	12	50
17	beta BHC	3.3	32.6	219	183	5	219	145	25	40
18	Bifenthrin	5.6	49.2	181	166	10	181	115	50	19
19	Bromfenvinfos	4.4	15.2	323	170	10	267	170	10	50
20	Bromfenvinfos methyl	4.2	21.1	295	170	10	295	109	10	2
21	Bromphos ethyl	4.4	17.4	331	316	14	329	314	14	99
22	Bromphos methyl	4.1	32.5	331	316	14	329	314	14	73
23	Bupirimate	4.7	21.5	273	193	5	273	108	5	54
24	Carbophenothion	5.1	29.7	342	157	10	199	143	10	78
25	Chlorbenside	4.3	15.2	125	99	18	125	89	16	36
26	Chlorfenapyr	4.7	38.2	247	227	15	247	200	25	84
27	Chlorfenson (Ovex)	4.5	13.0	175	111	10	111	75	14	100
28	Chlorfenvinphos	4.2	22.3	295	267	5	267	81	40	66
29	Chlorpyrifos methyl	3.6	60.2	286	271	26	288	93	26	50
30	Chlorpyrifos	4.0	65.9	314	286	5	314	258	5	31
31	Chlorthiophos	4.9	29.8	325	269	12	269	205	14	72
32	cis Chlordane	4.4	11.7	373	301	10	373	266	20	34
33	cis Nonachlor	5.0	15.3	409	300	18	263	193	28	82
34	cis Permethrin	6.8	74.1	183	153	15	163	128	5	6

Table A1. *Cont.*

				Quantifier Transition			Qualifier Transition			
	Compound Name	**RT, min**	**Dwell Time, ms**	**Ion1, a.m.u.**	**Ion 2, a.m.u.**	**CE1, eV**	**Ion1, a.m.u.**	**Ion2, a.m.u.**	**CE2, eV**	**Ion Ratio**
35	Coumaphos	7.1	98.9	362	109	15	210	182	15	49
36	Cyfluthrin	5.9	36.6	263	127	10	226	206	10	50
37	Cypermethrin	6.2	35.2	209	141	20	163	127	20	5
38	Cyprodinil	4.1	18.2	224	208	15	224	197	20	29
39	delta BHC	3.4	32.6	219	183	10	219	145	20	40
40	Deltamethrin	10.1	99.2	253	172	5	253	93	5	67
41	Diazinon	3.3	73.9	304	179	15	137	84	15	68
42	Dieldrin	4.5	14.5	345	263	8	279	243	8	50
43	Edifenphos	5.2	44.8	310	201	8	310	173	14	28
44	Endosulfan ether	3.5	38.8	241	206	14	239	204	12	165
45	Endosulfan I	4.4	12.2	239	204	15	195	160	5	165
46	Endosulfan II	4.8	17.3	207	172	15	195	159	10	24
47	Endosulfan sulfate	5.2	20.1	387	289	5	272	237	15	22
48	Endrin	4.9	15.8	263	193	35	245	173	30	42
49	Endrin aldehyde	5.0	15.2	250	215	24	173	138	16	95
50	Endrin ketone	5.7	74.0	250	215	24	173	138	16	96
51	EPN	5.7	40.8	157	110	15	157	77	25	50
52	Ethion	4.9	44.8	231	175	5	231	129	25	84
53	Ethylan (Perthane)	4.7	18.5	223	179	20	223	167	12	87
54	Etofenprox	8.0	99.3	163	135	5	163	107	15	81
55	Fenamiphos	4.4	16.5	303	195	8	303	154	18	92
56	Fenarimol	6.4	99.0	219	107	10	139	111	15	58
57	Fenchlorphos (Ronnel)	3.7	34.1	285	270	15	285	240	30	80
58	Fenitrothion	3.7	43.5	277	260	5	277	109	20	55
59	Fenson	4.1	23.9	141	77	8	77	51	14	19
60	Fenthion	3.9	35.2	278	169	20	278	109	20	43
61	Fenvalerate	8.9	99.1	167	125	12	125	89	20	69
62	Fipronil	4.3	13.3	213	178	10	213	143	20	6
63	Flucythrinate	8.1	99.1	199	157	5	157	107	15	87
64	Fludioxonil	4.5	19.3	248	154	25	248	127	30	46
65	Fluridone (Sonar)	8.5	99.1	328	154	10	310	154	10	50
66	Flusilazole	4.6	21.1	233	165	20	233	152	20	55
67	Flutriafol	4.5	18.5	219	123	12	219	95	20	54
68	Folpet	4.3	13.8	260	130	14	260	95	20	14
69	gamma BHC (Lindane)	3.4	28.4	219	183	5	219	145	5	16
70	Heptachlor	3.7	50.8	272	237	10	272	143	40	96
	Heptachlor epoxide (isomer B)	4.2	18.7	217	182	22	183	119	25	78
71	Hexazinone	5.3	43.6	171	128	5	171	83	10	10
72	Iodofenphos	4.5	16.6	377	125	10	377	109	15	50

Table A1. *Cont.*

				Quantifier Transition			Qualifier Transition			
	Compound Name	**RT, min**	**Dwell Time, ms**	**Ion1, a.m.u.**	**Ion 2, a.m.u.**	**CE1, eV**	**Ion1, a.m.u.**	**Ion2, a.m.u.**	**CE2, eV**	**Ion Ratio**
73	Iprodione	5.9	74.0	314	245	10	314	56	20	87
74	Isazophos	3.4	74.0	257	162	5	161	119	5	12
75	Isodrin	4.2	20.8	193	157	20	193	123	28	78
76	lambda Cyhalothrin	6.2	28.3	197	161	8	197	141	12	29
77	Lenacil	5.2	60.3	153	136	5	153	110	5	54
78	Leptophos	6.1	99.2	171	124	10	171	77	18	23
79	Malathion	3.8	34.1	173	99	15	158	125	15	18
80	Methacrifos	3.3	60.3	208	180	8	208	110	19	12
81	MGK-264	4.1	24.5	164	98	10	164	93	10	65
82	Mirex	6.4	99.1	274	239	14	272	237	14	62
83	Myclobutanil	4.6	18.8	179	152	5	179	125	10	14
84	Paclobutrazol	4.4	17.3	236	167	20	236	125	10	6
85	Penconazole	4.2	15.0	248	192	15	248	157	25	76
86	Pentachlorothioanisole	3.9	30.2	296	263	5	296	246	5	7
87	Phenothrin	5.9	36.6	183	153	15	123	81	8	22
88	Phosalone	6.1	73.9	367	182	5	182	138	8	50
89	Phosmet	5.7	60.3	160	133	15	160	77	30	50
90	Pirimiphos ethyl	4.1	74.1	333	180	5	318	180	5	0.2
91	Pirimiphos methyl	3.8	43.6	305	180	5	290	151	15	87
92	Procymidone	4.3	13.8	283	255	8	283	96	8	47
93	Profenofos	4.6	36.8	337	309	5	337	267	15	16
94	Prothiofos	4.5	22.5	309	239	15	309	221	25	79
95	Pyraclofos	6.5	40.7	360	194	5	360	138	5	50
96	Pyrazophos	6.4	65.8	221	193	10	221	149	15	31
97	Pyridaphenthion	5.6	65.9	340	199	5	340	188	5	17
98	Pyrimethanil	3.3	43.6	198	156	25	198	118	25	58
99	Pyriproxyfen	6.1	74.1	136	96	10	136	78	20	63
100	Quinalphos	4.3	99.1	157	129	15	146	91	30	44
101	Resmethrin	4.2	40.8	171	143	6	171	128	12	18
102	Sulfotepp	3.4	40.8	238	146	10	202	146	10	21
103	Sulprofos	5.0	26.4	322	156	10	156	141	14	35
104	tau Fluvalinate	9.2	99.1	250	200	20	250	55	15	47
105	Tebuconazole	5.3	60.3	250	153	12	250	125	20	18
106	Tefluthrin	3.3	74.2	177	137	15	177	127	15	32
107	Terbacil	3.3	57.5	161	117	5	160	118	5	24
108	Terbufos	3.5	36.6	231	175	10	231	129	25	50
109	Terbuthylazine	3.2	60.2	214	173	5	173	132	5	14
110	Tetrachlorvinphos	4.4	16.0	329	109	25	329	79	35	28
111	Tetradifon	6.0	74.1	356	229	10	356	159	10	90
112	Tetramethrin	5.6	49.2	164	123	5	164	81	10	0.2
113	Tolclofos methyl	3.7	34.1	265	250	15	265	220	25	52

Table A1. *Cont.*

				Quantifier Transition			Qualifier Transition			
	Compound Name	**RT, min**	**Dwell Time, ms**	**Ion1, a.m.u.**	**Ion 2, a.m.u.**	**CE1, eV**	**Ion1, a.m.u.**	**Ion2, a.m.u.**	**CE2, eV**	**Ion Ratio**
114	trans Chlordane	4.4	13.6	373	301	10	373	266	20	51
115	trans nonachlor	4.5	12.0	409	300	18	237	143	24	53
116	trans Permethrin	6.9	74.1	183	153	15	163	128	5	7
117	Transfluthrin	3.6	49.2	163	143	14	163	91	12	97
118	Triadimefon	4.0	44.2	208	181	5	208	127	15	46
119	Triadimenol	4.2	13.3	168	112	4	168	70	10	88
120	Triflumizole	4.3	12.9	278	73	6	278	55	12	50
121	Vinclozolin	3.6	74.2	212	172	15	212	109	40	78

Appendix B

Table A2. Description of the samples of the Saudi rice measured in this work. Imported Saudi rice samples (n = 20) were collected from Riyadh markets in 2022. Locally grown rice (Hasawiya rice) was collected from rice farms and the market of Al-Qurain village in Al-Ahsa Governorate, Eastern Province of Saudi Arabia (n = 10) in 2022 when rice is grown in the Kingdom of Saudi Arabia.

	Origin	Rice Type	Crop Year
SR1	India	Yellow long grain	2019
SR2	India	Yellow long grain	2021
SR3	India	Yellow long grain	2020
SR4	India	White long grain	2021
SR5	India	White long grain	2020
SR6	India	White long grain	2020
SR7	India	White long grain	2019
SR8	Al Hasa (Saudi Arabia)	Red medium grain	2020
SR9	Australia	White short grain	2021
SR10	Al Hasa (Saudi Arabia)	Red medium grain	2020
SR11	Pakistan	White long grain	2020
SR12	Thailand	White medium grain	2021
SR13	India	Yellow long grain	2021
SR14	India	Yellow long grain	2020
SR15	India	Yellow long grain	2021
SR16	India	White long grain	2020
SR17	Al Hasa (Saudi Arabia)	Red medium grain	2020
SR18	USA	Whit medium grain	2021
SR19	India	brown long grain	2019
SR20	Al Hasa (Saudi Arabia)	Red medium grain	2021
SR21	Al Hasa (Saudi Arabia)	Red medium grain	2021
SR22	Al Hasa (Saudi Arabia)	Red medium grain	2019
SR23	Al Hasa (Saudi Arabia)	Red medium grain	2019
SR24	India	White long grain	2021
SR25	India	White long grain	2021
SR26	Al Hasa (Saudi Arabia)	Red medium grain	2022
SR27	Al Hasa (Saudi Arabia)	Red medium grain	2022
SR28	USA	Yellow medium grain	2020
SR29	Al Hasa (Saudi Arabia)	Red medium grain	2019
SR30	India	White long grain	2021

Appendix C

Table A3. Description of the samples of the Bangladesh rice measured in this work. All rice samples were collected from the Tangail district (n = 25). They were collected from local markets as well as the local farmers.

ID	Origin	Rice Type	Crop Year
BR1	Rice Mill/Market	White medium grain	2022
BR2	Farmers	White medium long grain	2022
BR3	Rice Mill/Market	Off-white small grain	2022
BR4	Farmers	Off-white small grain	2022
BR5	Farmers	Light brown medium grain	2022
BR6	Farmers	Off-white medium grain	2022
BR7	Farmers	Off-white medium grain	2022
BR8	Farmers	Light brown small grain	2022
BR9	Rice Mill/Market	White small grain	2022
BR10	Rice Mill/Market	Off-white medium grain	2022
BR11	Farmers	Yellowish white long grain	2022
BR12	Farmers	Off-white medium grain	2022
BR13	Farmers	Off-white small grain	2022
BR14	Rice Mill/Market	White medium grain	2022
BR15	Rice Mill/Market	White medium grain	2022
BR16	Farmers	Off-white medium grain	2022
BR17	Farmers	Light brown small grain	2022
BR18	Farmers	Off-white medium grain	2022
BR19	Farmers	Off-white medium grain	2022
BR20	Farmers	Light brown small grain	2022
BR21	Rice Mill/Market	Light brown small grain	2022
BR22	Rice Mill/Market	White small grain	2022
BR23	Farmers	Yellowish white medium grain	2022
BR24	Farmers	Off-white medium grain	2022
BR25	Farmers	Off-white small grain	2022

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