



APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Analysis of Multi-Residue Pesticides in Rice by LC/MS/MS

Introduction

Rice is one of the most commonly consumed foods in the world. A

variety of pesticides have been used in rice production to control pests, weeds and diseases to increase crop yield. Pesticides applied in rice crops are often country/region specific due to the differences in legislation, weather and production system. Pesticide residue in rice not only affects the quality of the rice, but also threatens the health of general consumers. To prevent health risks, it is important to monitor the presence of pesticides and regulate their levels in rice. Several countries including the United States, China, Brazil, India, Japan and European Union (EU) have established maximum residue levels (MRLs) of pesticides for food and feed including rice.¹⁻³ The EU MRLs for pesticide residues in rice mostly range from 10 µg/kg to 8000 µg/kg depending on the pesticide.¹ To determine low levels of pesticides in rice, highly sensitive, selective and accurate analytical methods are needed. Due to the large number of pesticides potentially used in rice production, the use of multi-residue methods capable of determining many pesticides in one single run is the most efficient approach. Traditionally, pesticide residues were analyzed mainly by gas chromatography/mass spectrometry (GC/MS) methods,^{4,5} but GC is not a suitable technique for ionic and polar compounds, especially for compounds that are thermally labile in the GC injection port. Liquid chromatography tandem mass spectrometry (LC/MS/MS) has become the method of choice for pesticide analysis due to its high selectivity and sensitivity as well as its suitability for a wide range of compounds in various sample matrices.⁶⁻¹⁰

QuEChERS extraction method has been widely applied for analysis of multi-residue analytes in food samples including rice.^{4,8,9,10} In this study, a fast, sensitive and selective multi-residue method has been developed for analysis of over 200 pesticides in rice samples by coupling a modified QuEChERS extraction method with LC/MS/MS. Using time-managed-MRM™ in the QSight® triple quadrupole mass spectrometer, the optimum dwell time of multiple MRM transitions can be generated automatically for the targeted analytes. This not only saves time in method development but also improves data quality and analytical performance, as demonstrated in this study by the results of multi-residue pesticide analysis in rice samples.

Experimental

Hardware/Software

Chromatographic separation of pesticides was conducted by a PerkinElmer UHPLC System and analyte determination was achieved using a PerkinElmer QSight 220 triple quadrupole mass detector with a dual ionization source. All instrument control, data acquisition and data processing was performed using Simplicity 3Q™ software.

Method

Sample Preparation

Pesticide standards were obtained from ULTRA® Scientific (North Kingstown, RI). Rice samples were purchased from local grocery stores in Ontario, Canada. Different rice samples such as brown rice, black rice and white rice (including Jasmine, Basmati and Calrose) as well as two brands of organic rice samples were tested. These rice samples were originally produced in Thailand, Vietnam, India, Italy and the U.S. Rice samples were prepared according to a published procedure with minor modifications using QuEChERS kits (AOAC 2007.01 method) without dispersive SPE clean-up.¹⁰ One (1) µL of extract was injected directly onto the QSight LC/MS/MS system for quantification.

An organic brown rice sample was used as a controlled blank matrix. Recoveries from the rice sample matrix were evaluated by fortifications of pesticides at concentrations of 10 and 100 µg/kg. Calibration curves were built by eight levels of standards prepared in a neat solution (acetonitrile) and in the rice sample matrix (matrix-matched calibration). Matrix effects were evaluated by comparing the slopes of calibration curves obtained from the neat solution and rice sample matrix. To reduce false positives and negatives, at least two MRM transitions were monitored for each pesticide. LOQs (limits of quantification) were calculated based on a minimum S/N of 10 for both transitions.¹²

LC Method and MS Source Conditions

The LC method and MS source parameters are shown in Table 1. A partial list of the multiple reaction monitoring mode (MRM) transitions of the studied pesticides are shown in Table 2. The acquisition MS method is generated automatically by selecting the pesticides of interest from the built-in compound library in the time-managed-MRM module of the Simplicity software, including both positive and negative analytes.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	Brownlee, SPP Phenyl-Hexyl, 100 x 2.1 mm, 2.7 µm
Mobile Phase A	5 mM ammonium formate in water
Mobile Phase B	5 mM ammonium formate in methanol
Mobile Phase Gradient	Start at 10% mobile phase B and hold it for 1 min., then increase B to 95% in 15 min. and keep at 95% B for 2 min. Finally equilibrate the column at initial condition for 3 min.
Column Oven Temperature	40 °C
Auto Sampler Temperature	15 °C
Injection Volume	1.0 µL
MS Source Conditions	
ESI Voltage (Positive)	5000 V
ESI Voltage (Negative)	-4000V
Drying Gas	140
Nebulizer Gas	350
Source Temperature	325 °C
HSID Temperature	200 °C
Detection mode	Time-managed MRM™

Results and Discussion

Analytical Challenges for Multi-residue Pesticides

Analysis in Food Samples

Since the pesticides tested in this study contain both polar and non-polar compounds, to extract all the analytes from sample matrices, acetonitrile, an organic solvent, was used. However, the reverse phase LC method used aqueous mobile phase at the beginning of the LC run to retain the polar compounds on the column. Injecting a larger volume of organic solvent such as an acetonitrile sample extract on the LC would lead to poor chromatographic peaks for early eluting polar compounds. To overcome this problem, small sample volume was injected in this study.

Traditional MRM method development is not suitable for analysis of a large number of analytes such as hundreds of pesticide residues in a single run. It is both time-consuming and labor intensive to input all the mass transitions to a method manually. In addition, the dwell time for each transition cannot be optimized easily by traditional method. Therefore, a time-managed-MRM was applied for method development in this study to improve efficiency, data quality and method performance.

Sample matrix effect is the main concern for LC/MS/MS method development, especially for food analysis due to the diversity and complexity of food sample matrices. To overcome sample matrix effects, several approaches have been used, such as sample dilution, use of stable isotope internal standards, matrix-matched calibration, standard addition, sample clean-up, use of high efficiency columns for improved separation, and the use of alternative ionization sources.¹¹ In this study, sample matrix effects were evaluated by comparing the slopes (X) of calibration curves obtained from standards prepared in solvent (neat

solution) with slopes (Y) obtained from standards prepared in the rice sample matrix. Sample matrix effect (%) can be calculated by the percentage difference between the slopes, i.e. $(Y-X) \times 100/X$. When the percentage of the difference between the slopes of the two curves is positive, there is a signal enhancement effect, whereas a negative value indicates signal suppression effect. As shown in Table 3 and Figures 1 and 2, sample matrix effects are compound dependent. For example, some pesticides, such as acephate and propiconazole, showed signal enhancement (positive values), while others, such as chlorpyrifos and tricyclazole, showed ion suppression (negative values). As shown in Table 3, sample matrix effects for most of the pesticides studied are less than 20% and thus, calibration curves built from neat solutions could be used for their quantification without significant error according to EU regulation.¹² However, significant ion suppression effects were observed for chlorpyrifos (-55%) and tebuconazole (-18%). Therefore, to overcome matrix effects and reduce variations in analytical results, matrix-matched calibrations were used in this study for quantification of all analytes.

Method Performance

All calibration curves built from both the neat solution and rice sample matrix (matrix-matched calibration) showed good linearity (0.1 to 200 ng/mL) with correlation coefficient (R^2) larger than 0.99 (see Figures 1 and 2 for typical examples of calibration curves).

The recoveries of pesticides were evaluated by spiking the analytes to the samples at two concentration levels of 10 and 100 $\mu\text{g}/\text{kg}$, respectively. As shown in Table 3, the recoveries of analytes ranged from 70% to 120% with $\text{RSD} < 20\%$ for most of the pesticides studied.

The limits of quantification (LOQs) were determined by taking into account the signals of both quantifier and qualifier ions ($S/N > 10$ for both) and ensuring that the product ion ratios were within 20% tolerance windows of the expected.¹² Most of the tested pesticides have LOQs ranging from 0.5 to 20 $\mu\text{g}/\text{kg}$, which are well below the EU MRLs.

Table 2. MRM Transitions (partial list of the 213 pesticides studied).

Compound Name	Polarity	Q1 Mass	Q2 Mass	CE	EV	CCL2
Acephate	Positive	184.1	143.1	-12	25	-29
Acephate-2	Positive	184.1	125.1	-25	25	-41
Acetamiprid	Positive	223.2	126.1	-30	25	-49
Acetamiprid-2	Positive	223.2	99.1	-56	25	-73
Azoxystrobin	Positive	404.1	372.1	-18	25	-57
Azoxystrobin-2	Positive	404.1	344.1	-34	25	-71
Buprofezin	Positive	306.2	201.1	-18	25	-47
Buprofezin-2	Positive	306.2	116.2	-24	25	-52
Chlorantranilprole	Positive	484	452.8	-20	25	-66
Chlorantranilprole-2	Positive	484	285.8	-18	25	-65
Chlorpyrifos	Positive	350	198	-20	25	-53
Chlorpyrifos-2	Positive	350	97	-32	25	-64
Clothianidin	Positive	250.1	169.1	-16	25	-39
Clothianidin -2	Positive	250.1	132.2	-26	25	-48
Cumyluron	Positive	303.1	185	-20	25	-48
Cumyluron-2	Positive	303.1	125	-43	25	-69
Fenbutatin-oxide	Positive	519.3	197	-67	25	-112
Fenbutatin-oxide-2	Positive	519.3	350.9	-50	25	-97
Fenobucarb	Positive	208	152	-12	25	-32
Fenobucarb-2	Positive	208	95	-19	25	-38
Fluopyram	Positive	397	173	-35	25	-71
Fluopyram-2	Positive	397	145	-70	25	-103
Halofenozide	Positive	331.1	275	-18	25	-49
Halofenozide-2	Positive	331.1	104.9	-25	25	-56
Imazail	Positive	297.1	201	-25	25	-52
Imazail-2	Positive	297.1	159.2	-31	25	-58
Imidachloprid	Positive	256.2	175.2	-26	25	-49
Imidachloprid-2	Positive	256.2	209	-18	25	-42
Isoprothiolane	Positive	291.1	231	-16	25	-44
Isoprothiolane-2	Positive	291.1	189	-28	25	-54
Malathion	Positive	331.1	127.1	-22	25	-53
Malathion-2	Positive	331.1	99.1	-24	25	-55
Methamidophos	Positive	142	124.9	-20	25	-32
Methamidophos-2	Positive	142	94.1	-20	25	-32
Piperonyl butoxide	Positive	356.2	177	-13	25	-47
Piperonyl butoxide-2	Positive	356.2	119	-37	25	-69
Pirimiphos-methyl	Positive	306.1	164.1	-28	25	-56
Pirimiphos-methyl-2	Positive	306.1	108.1	-40	25	-67
Profenophos	Positive	375	304.8	-50	25	-75
Profenophos-2	Positive	375	346.8	-42	25	-113
Propiconazole	Positive	342.1	159.1	-42	25	-72
Propiconazole-2	Positive	342.1	69.1	-26	25	-58
Tebuconazole	Positive	308	70	-30	25	-58
Tebuconazole-2	Positive	308	125	-50	25	-76
Thiamethoxam	Positive	292	181	-28	25	-54
Thiamethoxam-2	Positive	292	211	-18	25	-45
Triazophos	Positive	314.1	161.9	-22	25	-51
Triazophos-2	Positive	314.1	118.9	-50	25	-76
Tricyclazole	Positive	190	163	-28	25	-44
Tricyclazole-2	Positive	190	136	-36	25	-51
Trifloxystrobin	Positive	409	186	-26	25	-64
Trifloxystrobin-2	Positive	409	206	-20	25	-59
Fludioxonil	Negative	246.6	125.9	40	-25	60
Fludioxonil-2	Negative	246.6	179.9	39	-25	60

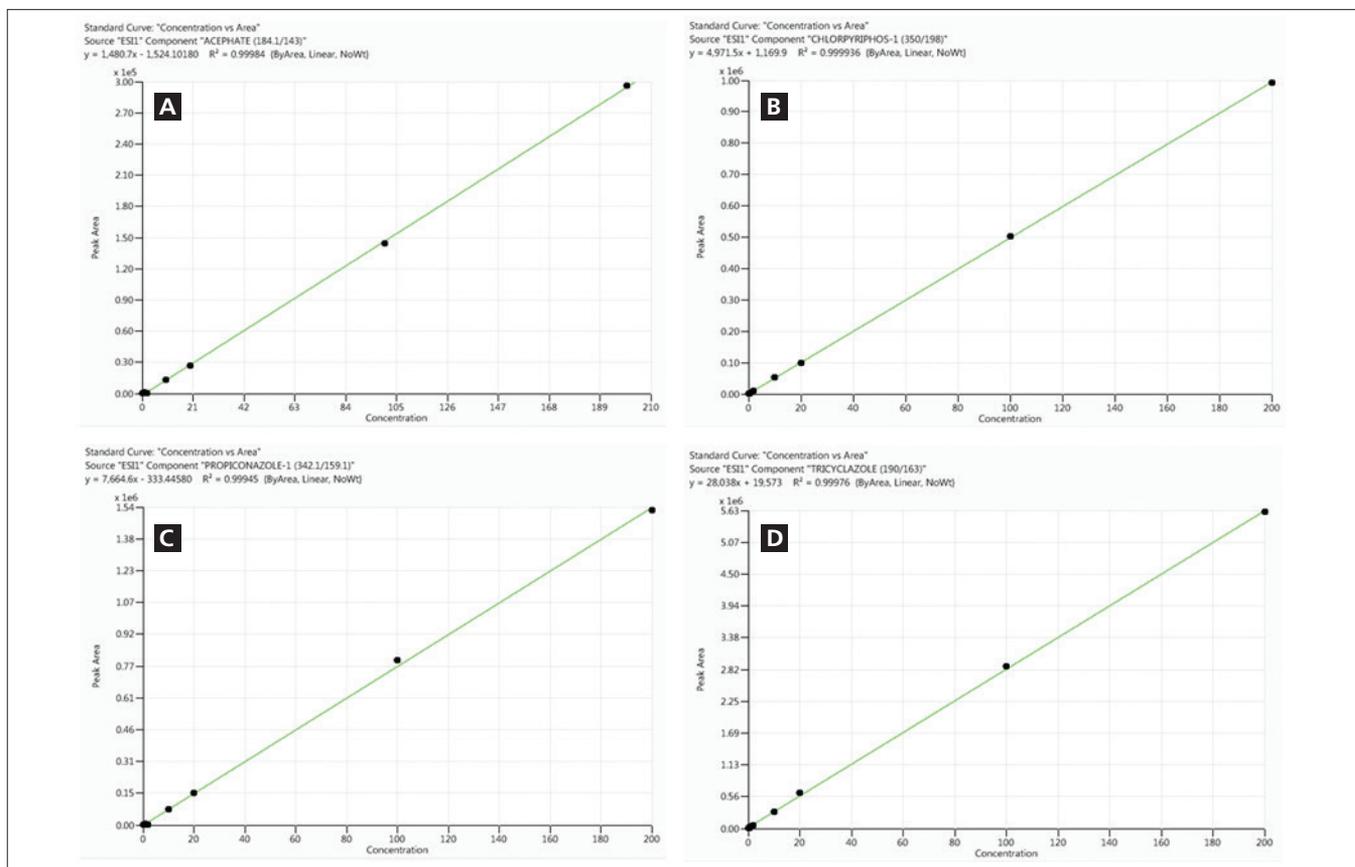


Figure 1. Calibration curves for acephate (A), chlorpyrifos (B), propiconazole (C) and tricyclazole (D) obtained from standards prepared in neat solutions (analyte concentrations range from 0.1 to 200 ng/mL).

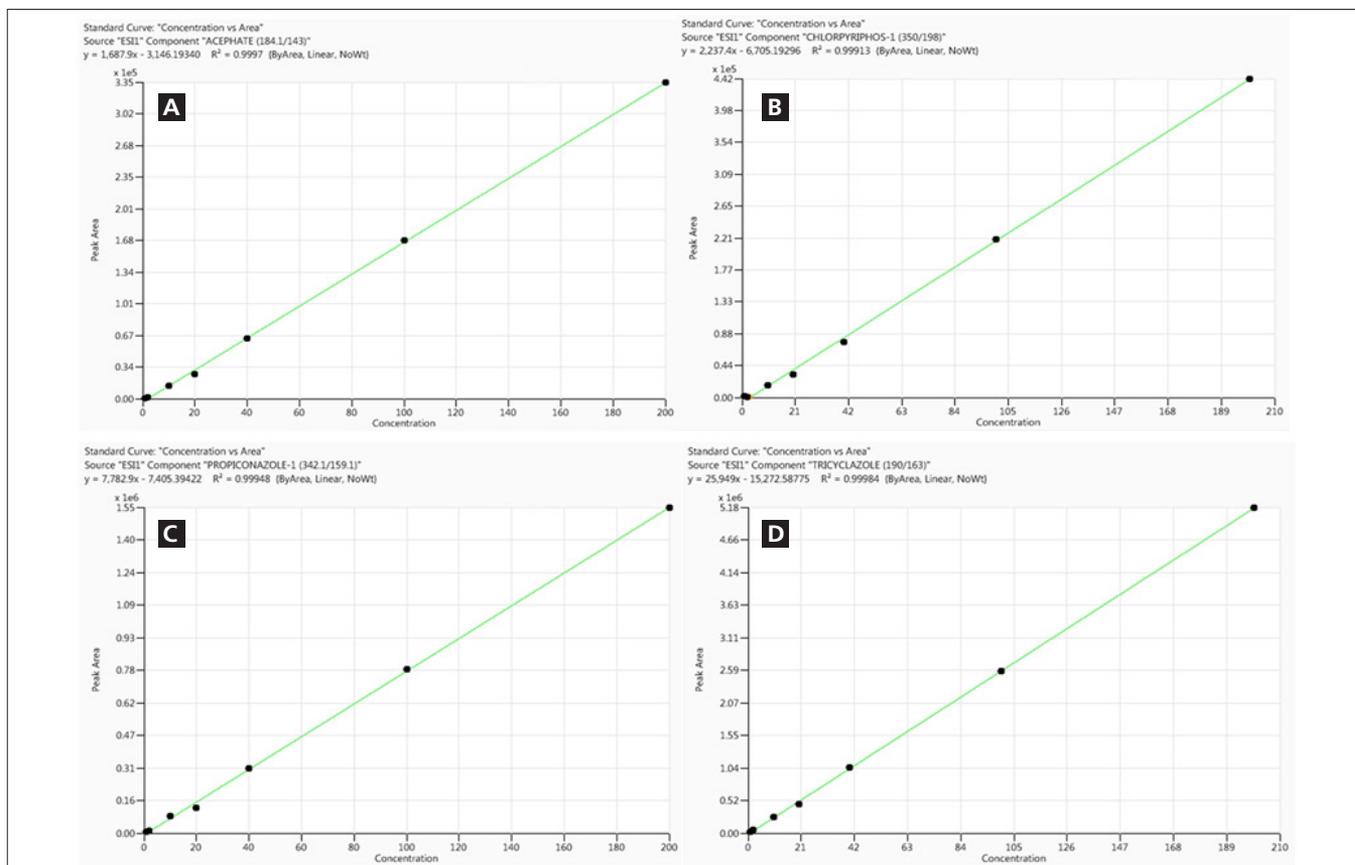


Figure 2. Calibration curves for acephate (A), chlorpyrifos (B), propiconazole (C) and tricyclazole (D) obtained from standards prepared in rice sample matrix (analyte concentrations range from 0.1 to 200 ng/mL).

Table 3. Results of retention time, recovery, reproducibility (%RSD), matrix effect and linearity for the most commonly detected pesticides in rice samples.

Pesticide	Retention Time (min)	% Recovery(%RSD) at 10 µg/kg	% Recovery (%RSD) at 100µg/kg	Matrix Effect (%)	Correlation Coefficient (R ²)
Acephate	1.88	101.1 (11.8)	81.9 (4.3)	14.0	0.9997
Acetamiprid	8.15	106.5 (2.6)	98.7 (2.3)	2.7	0.9996
Buprofezin	15.05	103.3 (2.9)	98.8 (3.5)	-3.1	0.9996
Chlorpyrifos	15.54	109.6 (10.4)	98.7 (5.0)	-55.0	0.9991
Clothianidin	6.70	105.7 (5.9)	111.2 (8.6)	17.0	0.9995
Cumyluron	12.74	98.9 (7.2)	96.1 (2.5)	-2.6	0.9984
Fenbutatin-oxide	16.90	69.5 (18.6)	78.8 (12.7)	13.1	0.9997
Fenobucarb	11.20	101.6 (2.9)	94.8 (1.9)	2.6	0.9976
Fluopyram	13.00	104.8 (3.6)	101.1 (3.1)	-2.7	0.9991
Halofenozide	12.26	89.4 (15.2)	88.3 (11.4)	-4.4	0.9980
Imazalil	14.33	89.6 (13.6)	95.3 (4.1)	-6.1	0.9996
Imidacloprid	7.57	77.5 (10.8)	112.2 (7.9)	-5.7	0.9991
Isoprothiolane	13.01	111.5 (2.7)	101.1 (2.3)	-0.4	0.9983
Malathion	13.25	92.0 (12.0)	86.0 (4.3)	-9.9	0.9995
Methamidophos	1.41	82.8 (10.1)	76.4 (14.3)	13.3	0.9978
Piperonyl Butoxide	15.26	106.0 (5.0)	105.2 (3.4)	-6.3	0.9977
Pirimiphos-methyl	14.71	107.5 (3.7)	98.8 (5.3)	-0.1	0.9997
Profenophos	14.82	110.7 (6.9)	103.0 (6.5)	-2.5	0.9988
Propiconazole	14.32	106.6 (7.1)	98.3 (2.8)	1.5	0.9994
Tebuconazole	13.72	102.2 (6.9)	104.2 (5.5)	-18.9	0.9993
Thiamethoxam	6.43	116.4 (10.0)	114.0 (14.9)	1.9	0.9991
Triazophos	13.46	117.8 (5.7)	99.5(3.0)	2.7	0.9979
Tricyclazole	9.27	84.2 (5.8)	80.7 (7.8)	-7.5	0.9998
Trifloxystrobin	14.91	106.7 (2.4)	106(4)	-5.8	0.9991

Sample Analysis

The developed method was applied for the analysis of pesticide residues in different food samples, including eleven rice samples; one wheat sample and one veggie straw sample. Figure 3 showed the overlapped MRM chromatograms of pesticides identified and quantified from a brown rice sample. Table 4 lists the pesticide residues determined in the eleven rice samples and the EU MRLs in µg/kg (NA*; some pesticides that are not included in the EU MRLs list were also determined by this method). As shown in Table 4, many of the pesticides identified from sample 4 (S4) and sample 10 (S10) are quite similar because these two rice samples were produced from the same region, which indicates that pesticides applied to rice crops during production are country or region specific due to the regulation and weather conditions in that region.

Conclusion

A LC/MS/MS method for multi-residue pesticides analysis in rice was developed by coupling a UHPLC system to a Q-Sight 220 triple-quadrupole mass spectrometer. The method can be applied for the analysis of over 200 pesticides in rice with LOQs well below the limits set by regulatory agencies. The time-managed-MRM module has simplified the creation of MS method with optimum dwell time for monitoring a large number of analytes in food samples. The QuEChERS

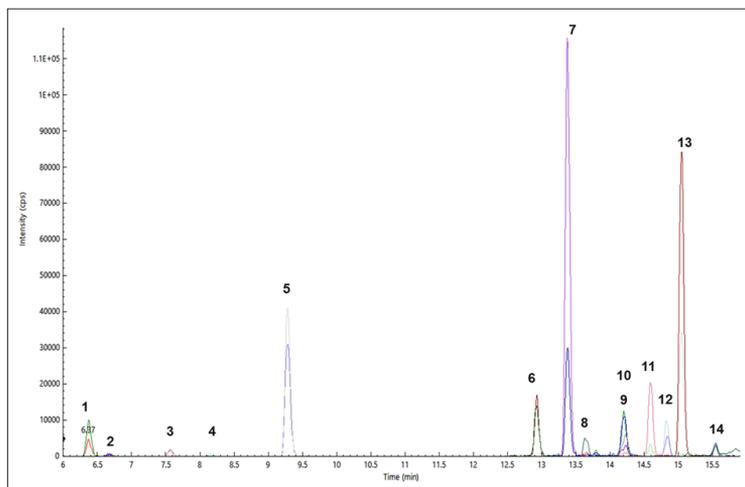


Figure 3. Pesticides determined from a brown rice sample (S10): thiamethoxam (1), clothianidin (2), imidacloprid (3), acetamiprid (4), tricyclazole (5), isoprothiolane (6), triazophos (7), tebuconazole (8), imazalil (9), propiconazole (10), profenophos (11), trifloxystrobin (12), buprofezin (13), and chlorpyrifos (14).

sample extraction utilized in this study demonstrated good recovery (70-120%) and reproducibility (RSD <20%) for most pesticides. The developed method showed excellent linearity with R² > 0.99 for all the studied pesticides in rice matrix. A number of pesticide residues were identified and quantified from eleven rice samples with concentrations at or below the EU MRLs. This LC/MS/MS method has also been applied for other food analyses such as wheat and veggie straws samples with good performance. The method presented here can be easily adapted for multi-analyte screening and quantification, providing a single method for more cost-effective analysis of pesticides in rice and other food samples.

Table 4. Pesticide residues determined from eleven rice samples (S1 to S11), in µg/kg.

Pesticide	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	MRL
Acephate				2.0								10
Acetamiprid							0.3			0.8		10
Buprofezin				9.1						46.5		500
Chlorpyrifos				0.5	0.3		1.4			8.7		50
Clothianidin				7.0						3.0		500
Fenobucarb				4.1								NA*
Fluopyram					0.5							10
Halofenozide						5.0						NA*
Imazalil	1.4			2.5		4.6				2.6	1.6	50
Imidacloprid				2.8			1.1			9.2		1500
Isoprothiolane				4.4	9.3		2.9			14.7		5000
Malathion							1.8		2.2			8000
Methamidophos				0.5								10
Piperonyl Butoxide		0.6	1.3					0.8				NA*
Pirimiphos-methyl			1.4									500
Profenophos										5.2		10
Propiconazole				8.3	8.4	6.7	4.1			18.1		1500
Tebuconazole				5.9	5.2		0.9			12.0		1000
Thiamethoxam				10.6						11.0		10
Triazophos				0.6			0.5			17.6		20
Tricyclazole			16.4	5.8	7.6	20.6	0.6			40.2		1000
Trifloxystrobin										1.6		5000

NA*: pesticides not listed in the EU MRLs database, but can be determined by this method.

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