

# ANALYSIS OF PESTICIDE RESIDUES IN BROWN RICE USING MODIFIED QuEChERS MULTIRESIDUE METHOD

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## Introduction

The occurrence of pesticides not only affects the quality of rice but also threaten human health and the environment. Therefore, monitoring of pesticides residues in rice is an important issue to ensure food safety. However, the complexity of the matrix and the low concentration of pesticides present in rice make it difficult for the analysis of pesticides. Despite technological improvements in analytical tools, pre-treatment will be necessary prior to instrumental analysis to avoid interferences during the analysis of these compounds. Although there are several methodologies reported for the determination of pesticides in cereals, there are only a few for polished or unpolished rice especially using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Polarity of pesticides and type of matrices has a great impact on extraction of pesticides from food. Due to its simplicity, low cost, relatively high efficiency and minimal number of steps, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has been developed as an alternative for sample preparation for the analysis of multiple pesticide residues in fruits and vegetables. There are a lot of experimental works that have been published aiming to improve the overall performance or to apply QuEChERS specifically to some difficult issues of pesticide residue analysis adapting it as a template to user needs. Recently, several modifications have been introduced to the original method, aiming to improve the recoveries for some problematic pesticides or for the analysis of complicated matrices. High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has been proven to be a powerful technique for the analysis of drugs and pesticides at trace concentration levels owing to its high selectivity, precision, and sensitivity. Among several ionization modes, electrospray ionization (ESI) has been proven to be reliable, robust, and sensitive. The present study explored a HPLC-ESI-MS/MS method for the determination of pesticide multiresidues using modified QuEChERS approach and evaluated the method to extract pesticides from brown rice. The developed analytical method has been applied to real samples to provide scientific evidence for monitoring of pesticides in brown rice.

## Materials and methods

All pesticides were obtained from Dr. Ehrenstorfer GmbH (Augsberg, Germany). Gradient grade acetonitrile (MeCN) and glacial acetic acid (100%) were purchased from Merck KGaA (Darmstadt, Germany). Formic acid and ammonium acetate (>98 and 99% purity) were provided by Sigma Aldrich (St. Louis, MO). HPLC-MS/MS analysis was performed using an Agilent Technologies (Palo Alto, CA) model 1200 series HPLC coupled to an Agilent 6410 triple-quadrupole mass spectrometer. Instrument parameters were: gas temp 350 °C, gas flow 10 Lmin<sup>-1</sup>, and nebulizer 50 psi. N<sub>2</sub> gas was used as nebulizer gas. Ionization was done by electrospray ionization at positive mode. Standard mixtures of 72 pesticides and internal standard, triphenylphosphate (TPP) were initially determined for precursor and product ions, mass spectra, retention times, and optimum voltages for fragmentor and collision cell at positive ionization mode using full scan. Acquisition time, flow rate and gradient were also optimized using standard mixtures at multiple reactions monitoring (MRM) mode. Paddy rice samples were subjected to primary milling operation to remove husk. The resulting brown rice samples were grinded in the grinder (Foss Analytical, Seoul). Pounder rice samples were kept in the freezer at -20 °C until used. A 5 g of pounder samples were weighed into 50 mL empty polypropylene centrifuge tubes. A 100 µL of desired concentration of pesticide mixture was spiked onto sample for recovery test samples and 100 µL of acetonitrile for matrix blanks and screening samples respectively. A 100 µL of 10 mg L<sup>-1</sup> TPP standard was spiked to all samples. The tubes were stood for 1 hour to assure the penetration of pesticides and TPP into sample matrix. Then, 10 mL each of deionized water and 1 % formic acid in acetonitrile were added into the centrifuge tubes. Capped centrifuge tubes were horizontally laid down on the reciprocating shaker and shaken for 1 hour. After that, 4 g anhydrous MgSO<sub>4</sub> and 2 g NaCl were added into vials and shaken vigorously by hand for 2 minutes. Centrifugation at 3500 rpm was conducted to achieve solvent separation. Extract clean-up was done by vortex

mixing 1 mL of supernatant with 150 mg anhydrous  $\text{MgSO}_4$ , 50 mg PSA, and 50 mg  $\text{C}_{18}$ . After centrifugation at 12000 rpm, 0.5 mL of supernatant was thoroughly mixed with 100  $\mu\text{L}$  standard for matrix match calibration standards and with 100  $\mu\text{L}$  acetonitrile for the rest of the samples. The final sample extract was filtered with 0.2  $\mu\text{m}$  PTFE filter and injected onto HPLC-MS/MS. Schematic flow chart diagram regarding extraction and cleaning was presented in Fig. 1. All the rice samples were screened for pesticides using above sample preparation method. The samples free from pesticides were selected to use as control and recovery test sample. For extraction with acetonitrile following previously reported method, acetonitrile was used as extraction solvent. Solvent separation and clean-up procedures were the same as described in above procedure. For extraction with acetic buffer, 1% acetic acid in acetonitrile was used as extraction solvent. Except the changes in extraction solvent, the whole procedure was the same as described above.

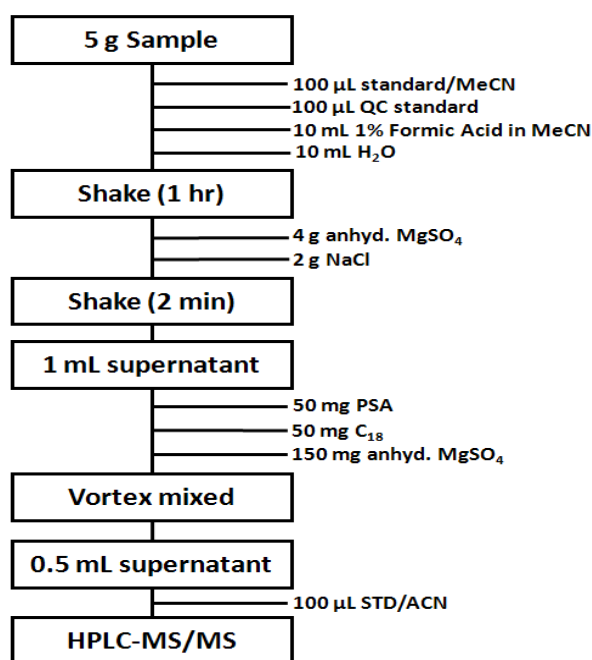


Figure 1. Schematic diagram of sample preparation method

## Results and discussion

The experiment was primarily aimed to monitor residual pesticides in brown rice using previously reported method. The method involved 10 mL acetonitrile as extraction solvent together with the same volume of water to assure swelling of dry rice sample. However recoveries of sulfonylurea herbicides among target pesticides (total = 72) were found to be very low and invalid as can be seen in Fig. 2. Sulfonylurea herbicides are weak acid (pKa from 3 to 5). They are negatively charged in neutral and alkaline condition. PSA that used for cleaning-up the sample extract is weak anion exchanger so that negatively charged pesticides can interact with the sorbent due to their chemical nature. This chemical interaction may result in lower recovery of sulfonylurea pesticides. To avoid low recovery of these pesticides, one research group did not perform dispersive SPE clean-up step. However, there have been proven that the use of PSA is very effective to remove sample co-extractives such as carboxylic acids in the fatty sample like rice.  $\text{C}_{18}$  is non-polar sorbent that more effectively retains trace amounts of lipids from the extract of fatty matrices. Without using PSA and  $\text{C}_{18}$ , greater matrix effect induced by fatty sample co-extractive will obviously interfere chromatographic results. In addition, occurrence of lipids and fatty co-extractive can reduce durability of HPLC column. Therefore, the method in which dispersive SPE clean-up is included and high recovery of sulfonylurea pesticides is achieved should be developed.

On the other hand, a method to extract phenoxy acid herbicide from rice matrix using 5% formic acid. The recoveries of pesticides were sufficiently high. The authors claimed that phenoxy acid analytes would be pushed towards their protonated neutral forms by the addition of formic acid into solvent extraction. This basic idea of

acidification of solvent was adapted for the development of QuEChERS based sample preparation method to extract target pesticides including sulfonylurea herbicides from rice matrix. Extraction solvent (MeCN) was acidified using 1% acetic acid or 1% formic acid. The recoveries of sulfonylurea herbicides (4 out of 7) are still low with 1% acetic acid (Fig. 2). But, the recoveries of those herbicides are found to be satisfactory with 1% formic acid (Fig. 3). Sulfonylurea herbicides show significant matrix effect in both matrix-matched standards (MMS) with respect to standards in acetonitrile, except bensulfuron-methyl. The calibration curve of bensulfuron-methyl in both types of extract and in acetonitrile solvent is not significantly different. MMS of flucetosulfuron and imazosulfuron in extract with reported method are significantly different from standard in acetonitrile while MMS in extract with modified method shows no difference. However, there are no significant differences between MMS with reported method and modified method for all herbicides when their calibration curves are statistically compared. This reveals that the cleanness of sample extract with modified method is comparable to reported method and the recoveries of sulfonylurea herbicides are better and satisfactory with modified method.

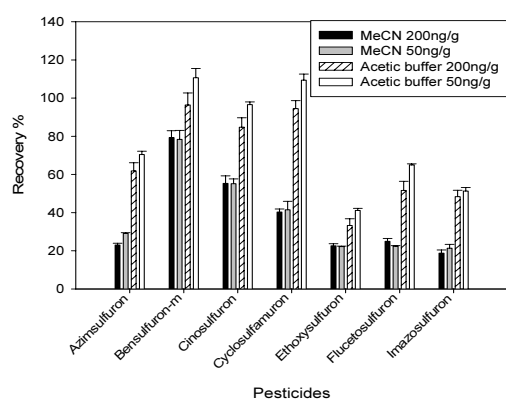


Figure 2. Recoveries of sulfonylurea herbicides from brown rice sample extracted with MeCN only and 1% acetic acid in MeCN

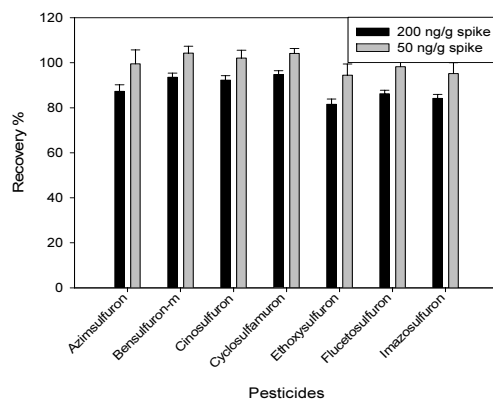


Figure 3. Recoveries of sulfonylurea herbicides from brown rice sample extracted with 1% formic acid in MeCN

In order to evaluate the effectiveness and applicability of the developed method, real samples collected from various places of republic of Korea were analyzed for the determination of residues of pesticides. Total number of 40 samples was collected and each sample was analyzed in duplicate using the developed method. The results are reported in Table 1. A total of 22 pesticides are observed in the 31 brown rice samples. Among them, fungicide tricyclazole is the most frequently found pesticide followed by etofenprox, ferimzone, and hexaconazole in decreasing order of observed frequency. The concentration of those pesticides varied between 0.001 and 1.168 mg kg<sup>-1</sup>.

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No	Pesticide	Frequency observed	Conc. range (mg kg <sup>-1</sup> )	MRL (mg kg <sup>-1</sup> )
1	Azoxystrobin	3	0.002–0.015	1
2	Bensulfuron-methyl	2	0.005-0.006	0.02
3	Cafenstrole	2	0.0003-0.008	0.05
4	Carbendazim	2	0.001-0.002	0.05
5	Carpropamid	1	0.022	1
6	Dinotefuran	2	0.026-0.017	1
7	Edifenphos	2	0.021-0.035	0.2
8	Etofenprox	8	0.001-0.296	1
9	Fenoxaprop-ethyl	3	0.001-0.0015	0.05
10	Ferimzone	7	0.009-0.448	0.7
11	Hexaconazole	6	0.002-0.085	0.3
12	Imidacloprid	1	0.013	0.05
13	Indoxacarb	1	0.006	0.1
14	Iprobenfos	5	0.002-0.019	0.2
15	Methoxyfenozide	4	0.004-0.008	1
16	Orysastrobin	1	0.019	0.3
17	Pencycuron	4	0.003-0.005	0.3
18	Propiconazole	1	0.057	0.1
19	Tebuconazole	1	0.011	0.05
20	Thifluzamide	1	0.091	0.1
21	Tricyclazole	18	0.003-1.168*	0.7

Table 1. Pesticides observed in real rice samples along with frequency existed, observed concentration range, and their MRLs regulated for rice in republic of Korea.

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