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# Validation of an HPLC-MS/MS Method to Determine Pesticides Residues in Rice

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**Abstract:** For the global human population, rice is the most significant and primary cereal crop. There is an increasing interest in the development methodologies to detect pesticides residues in food and feed samples. In the present study, a method was developed and validated to determine 121 pesticide residues in rice, according to the guidance document SANTE/11312/2021. QuEChERS was chosen as the pesticide extraction method and the extract was analyzed by high performance liquid chromatography tandem mass spectrometry. The methodology was shown to be precise and accurate and was applied to commercial samples of rice.

**Keywords:** Rice; Pesticide Residues; HPLC-MS/MS.

## 1. Introduction

The term "pesticides" refers to a class of chemicals that include those used as insecticides (which kill insects and other arthropods), fungicides (which kill fungi), herbicides (which kill weeds and other plants that grow in undesirable places), rodenticides (which control mice and other rodents), nematicides (which kill nematodes) and molluscicides (which kill snails and slugs).[1]. The following citation is the definition of pesticides provided by the FAO: "*Pesticide means any substance or mixture of substances or biological ingredients intended for repelling, destroying or controlling any pest or regulating plant growth*" [2]. Three million tons of pesticides are used worldwide every year, while only 1% of total pesticides are effectively used to control insect pests on target plants. This not only increases the cost of agricultural production but also affects the quality and safety of agricultural products and the ecological environment [3]. Recent research demonstrates that around 2 million tons of pesticides are utilized, with herbicides accounting for 47.5%, insecticides for 29.5%, fungicides for 17.5%, and other pesticides for 5.5% [4]. Pesticides are categorized using a variety of categories, including chemical classes, functional groups, modes of action, and toxicity. Chemical classifications divide pesticides' components into organic and inorganic groups. Inorganic pesticides include substances like copper, lime, sulfur, ferrous sulfate, and sulfate of copper. Organic pesticides have more complex ingredient lists. Chemical classifications for organic pesticides include metaldehyde molluscicides, metal phosphide rodenticides, carbamate insecticides, organophosphorus insecticides, carbamate herbicides, synthetic pyrethroid insecticides, metabolite and hormone analog

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herbicides, synthetic urea herbicides, triazine herbicides, benzimidazole nematocides, and vitamin D-based insecticides.

Different parameters, including specificity, concentration range, linearity, limit of quantification (LOQ), precision (repeatability, intra-laboratory reproducibility), and accuracy (using recovery tests), were used to validate the HPLC-MS/MS method. Additionally, expanded uncertainty was assessed.

Rice blank samples were spiked at 3 different levels (n=6), accounting for the maximum level (ML) of each pesticide residue, in order to determine repeatability ($RSD_r$) and intra-laboratory reproducibility ($RSD_R$). For the $RSD_R$ determination, trials were conducted over the course of three distinct days by various operators. Utilizing verified reference materials and recovery assays, the method's accuracy was determined.

## 2. Material and Methods

### *2.1. Extraction*

A QuEChERS procedure was used to extract pesticides from rice. First, weight 10 g of sample for a tube of 50 mL, add 20 mL of cold water, and stand by for 1 hour. Next, add 10 mL of acetonitrile (ACN) and vortex. For the liquid-liquid partitioning step, 6.5 g of an extraction salt mixture (4 g of magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate, and 0.5 g of disodium hydrogen citrate sesquihydrate) were added. They were vortexed for 1 minute, then centrifuged at 4000 rpm for 5 minutes. After centrifugation, 6 mL of the extract were added to a mixture with primary secondary amine bonded silica (PSA) and anhydrous magnesium sulfate (1.05 g), which corresponds to a clean-up step called dispersive solid-phase extraction. To 220 mL of ACN on the Eppendorf, 1 mL of the extract was added after mixing and centrifuging at 4500 rpm for 2 minutes. In a mini-uniprep™, 500 µl of the extract was combined with 25 µl of an internal standards solution. Electrospray ionization (ESI)-based triple quadrupole ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) was used to examine the extract.

### *2.2. Spiking Expirement*

Spiking studies were carried out to ascertain the target analytes' recovery rates. Using a multi-pesticide standard solution in acetonitrile (v/v), blank samples of rice (10 g) were spiked at three different concentrations (5,10, and 50 g/kg). After fortification, the solution was exposed to the matrix for 30 minutes at room temperature and in the dark. The extraction procedure was then carried out as explained in Section 2.1

## 3. Results

### *3.1. Validation of the Method*

A total of 121 pesticides were validated using the method in accordance with the standards outlined in SANTE/11312/2021, which specifies the validation requirements for the official regulation of pesticides in foods in the EU. [5].

By using matrix-matched calibration curves (mean of six curves) with different ranges for various pesticide residues, linearity was assessed. Depending on the pesticide, the linear range of the calibration curves was between 5-100, 10-100, or 50-100 g/L. The MRL of pesticide residues in rice was 5, 10, or 50 g/kg, which is sensitive enough to meet the requirements established by EU legislation. [6]. The determination coefficients varied between 0.9532 and 0.9983, indicating suitability for pesticides quantification. Recoveries for the 121 analyzed pesticides ranged between 70.0 and 119%, at 3 concentration levels. The specificity criteria were met for all pesticides at 5 µg/kg, except for chlorantraniliprole, chlorfenvinphos and metazachlor where they were met at 10 µg/kg and for hexythiazox and fludioxanil where they were met at 50 µg/kg. For the pesticides with LOQ of 5 µg/kg,

the mean of the three spiking levels (5, 10, and 50 μg/kg) was used to calculate precision, repeatability, and recovery. For the pesticides with LOQ of 10 μg/kg, the mean of levels 10 and 50 μg/kg, was used to calculate precision, repeatability, and recovery and for the pesticides with LOQ of 50 μg/kg, just the data achieved at this level are used present the same validation parameters.

All of the method's recoveries were within the parameters specified by the SANTE/11312/2021 criteria. Repeatability of the method was evaluated by the Relative Standard Deviation $RSD_r$. $RSD_r$ was between 5.71 and 17.1%. Reproducibility was evaluated by the Relative Standard Deviation $RSD_R$ at 3 different days of analysis, different concentration levels and values were considered acceptable (varied between 6.62 and 19.7%).

The expanded uncertainty ranged between 8% for fenamiphos sulfoxide and 49% for profenofos. Then, it was determined that, in accordance with the SANTE/11312/2021, the pesticide residue values do not need to be adjusted for recovery because the mean recovery is within the range of 70%-120% and the condition of 50% expanded measurement uncertainty is reached.

### *3.2. Pesticides Residues in Rice Commercial Samples*

Four commercial rice samples, one long grain rice, one basmati, one brown rice and one Carolino rice (short grain rice) were analyzed regarding their content in the 121 pesticide residues included in the UHPLC-MS/MS methods validated earlier. Rice samples were collected in June 2023 in local supermarkets. Sample 1 corresponds to long grain rice, sample 2 to basmati rice, sample 3 to Brown rice and Sample 4 to short-grain (carolino) rice. All samples are negative for pesticide residues, However, in a different study [7], Melo et al. (2020) discovered imidacloprid in 3 commercial rice samples, with concentrations of 0.0054–0.0008 mg/kg, 0.0125–0.0005 mg/kg, and 0.0658–0.0018 mg/kg, respectively. The contaminated sample 3 corresponds to parboiled rice, rice sample 2 to medium-grain rice, and rice sample 1 to basmati rice. No samples of this herbicide surpassed the MRL for rice, which is 1.5 mg/kg. [7].

According to Tran-Lam et al. (2021), the majority of pesticides found in commercial rice samples taken from Hanoi markets were insecticides, which were present in 14 out of the 20 samples. [8].

Tauseef et al., found in their study six rice samples contaminated with pesticides. Dimethoate, carbofuran, carbaryl, atrazine, triazophos, diazinon, bifenthrin, and hexaconazole were among the pesticides that exceeded EU-MRLs. [9].

Since 2015, several notifications have been reported through the Rapid Alert System for Food and Feed (RASFF). One notification is the presence of chlorpyrifos-methyl in rice from Pakistan found in Belgium, with a concentration of 0.039 mg/kg, where the MRL maximum is 0.01 mg/kg. Another one is the presence of thiamethoxam, tricyclazole (an unauthorized substance), and imidacloprid in rice from India found in Germany. With a concentration of 0.116 mg/kg, 0.207 mg/kg, and 0.026 mg/kg, respectively, and for which one the MRL is 0.01 mg/kg. Justifies the importance to carry out analyzes on commercial samples, for the safety of consumers.

## 4. Conclusions

Foods contaminated with pesticides residues and mycotoxins are associated with negative human health effects. It is therefore of utmost importance to develop simple and cost-

effective analytical methodologies to enhance food safety and prevent the possible harms caused by contaminants, which may be present in foods, in particular in cereals like rice. The analysis of pesticides residues in foods is of great importance, but also very challenging as requires the analysis of many compounds with different properties at very low levels in complex matrices. A method to determine pesticides residues in rice using QuEChERS extraction followed by UHPLC-MS/MS was successfully validated. The validation parameters were acceptable for 121 pesticides residues according to the guidelines at established in the European Union [5]. The proposed method was found to be selective, sensitive, precise, accurate, and cost-effective. Moreover it was applied to commercial samples acquired in portuguese supermarkets which revealed to be in accordance with EU legal limits for pesticides residues. This method will be of great interest to monitor pesticides residues in cereal samples.

**Supplementary Materials:** The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table A1: Results of the validation of the HPLC-MS/MS method to determine 121 pesticides in rice: determination coefficient ($r^2$) in matrix-matched curves, recovery, repeatability ($RSD_r$) and precision ($RSD_R$), limit of quantification (LOQ) and expanded uncertainty (U).

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