Development of a simple HPLC procedure for the determination of prochloraz residues in mushrooms

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ABSTRACT

A simple HPLC-UV procedure is described in our paper which is suitable for the rapid and cost-efficient determination of prochloraz in mushrooms. Prochloraz is the only fungicide in EU which use is allowed in mushroom production. The aim of our work was the development of a simple method that is suitable for the control of this pesticide in everyday analyses during mushroom production. The procedure involves a simple sample preparation method based on solid-liquid extraction (modified QuEChERS extraction method EN 15662) followed by an HPLC-UV determination (recovery: 97–99%; limit of detection LOD: 0.01 mg/kg; limit of quantification LOQ: 0.05 mg/kg).

KEYWORDS

pesticide analysis, prochloraz, liquid chromatography, cultivated mushrooms, Agaricus Bisporus

INTRODUCTION

The fungicide prochloraz, N-propyl-N-[2-(2,4,6-trichlorophenoxy)-ethyl]imidazole-1-carboxamide is used for the control of leaf scab and grey mold on fruits, vegetables and ornamentals worldwide. In EU, prochloraz has been released by the legislation for field application on cultivated mushrooms. Prochloraz is the only fungicide that is allowed in the EU to apply during the cultivation of mushrooms, the permitted exposure limit is 3 mg/kg. Some methods for the individual determination of prochloraz in food matrices have been previously reported using gas chromatography coupled to mass spectrometry (GC-MS) [1, 2], capillary electrophoresis [3], high-performance liquid chromatography (HPLC) [4, 5], and liquid chromatography coupled to tandem mass spectrometry (LC-MS, LC-MS-MS) [6, 7]. However a simple procedure for the determination of prochloraz in mushrooms is currently not available. The QuEChERS (quick, easy, cheap, effective, rugged and safe) method, which was developed by Anastassiades et al. [8], has proved to be an attractive pretreatment method for pesticide multiresidue analysis in fruits and vegetables. Nevertheless, in many analytical methods, the importance of interactions between factors is often not taken into account. Hence, conventional optimization strategies for analytical methods often fail to achieve exact specifications. Several complicated and expensive LC-MS and LC-MS-MS analytical procedures are described for the analysis of prochloraz that are not suitable for the on time analysis and everyday quality assurance of cultivated mushrooms. New Champignons Ltd. is one of Hungary’s most significant mushroom producers, the enterprise produces mainly Agaricus Bisporus, Pleurotus species and Lentinula edodes. In our study we focus on the development of a rapid and simple analytical procedure for the determination of prochloraz.
residues in cultivated mushroom. The procedure involves a modified QuEChERS extraction method followed by HPLC-UV detection, the effectiveness and applicability of the developed method was evaluated in real samples from mushroom cultivation.

EXPERIMENTAL

Reagents and standards

Samples. Cultivated and wild mushrooms were provided for the experiments by New Champignons Ltd., Hungary. Wild mushrooms were originated from different parts of Hungary near to intensive agricultural areas.

Chemicals and reagents. Prochloraz standard (>98.0% purity) was purchased from Sigma-Aldrich, Germany. QuEChERS Kits (Extraction Kit: EN 15662, Part No.: KSO-8909: 4.0 g magnesium sulfate, 1.0 g sodium chloride, 1.0 g SCTD, 0.5 g SCDS; dSPE Kit: KO-8924) were obtained from Phenomenex/GenLab. Formic acid, HPLC grade methanol and HPLC grade water were purchased from VWR International Ltd., while acetonitrile (HPLC grade) was provided by Fisher Scientific.

Sample preparation. Collected mushrooms were frozen to −22 °C, the hard, whole mushrooms (3–6 pieces) were blended. Mushroom pulp (10 g) was thoroughly shaken with acetonitrile (10 mL) in QuEChERS (50 mL) centrifuge tube for 1 min. QuEChERS EN 15662 Method Pucket mixture (magnesium sulfate 4 g, sodium chloride 1 g, trisodium-citrate-dihidrate 1 g, disodium-citrate-sesquihidrate 0.5 g) was added to the mixture, and it was intensively further shaken for 1 min. Sample was centrifuged for 5 min at 3,000 rpm, then 6 mL of the supernatant was put into a QuEChERS dSPE Kit PP centrifuge tube, which contained PSA (150 mg) and magnesium sulfate (900 mg). A thorough half min shaking was followed by centrifuging (5 min, 3,000 rpm). To 3 mL of the supernatant was added 30 μL 5 V/V% formic acid solution in acetonitrile. Sample was directly injected to HPLC apparatus.

HPLC measurement. HPLC measurements were carried out with a Dionex UltiMate 3000 HPLC apparatus coupled with Diode Array Detector (Thermo Scientific). Reversed phase column was applied (Hypersil Gold C18 column 150 mm × 4 mm, 3 μm), HPLC measurements were carried out in three parallel measurements, mobile phase was a methanol-water (70:30) mixture. Measurements were implemented with an isocratic eluent flow rate of 0.4 mL/min, with full loop (20 μL) injection. HPLC measurement lasts for 20 min, the characteristic prochloraz peak comes at 13.27 min retention time. Calibration: 0.05 μg/mL – 5.0 μg/mL (limit of detection LOD: 0.01 μg/mL; limit of quantification LOQ: 0.05 μg/mL). The peak at 13.27 min (R²) from mushroom extracts using the QuEChERS sample preparation method was identified and compared with the UV spectrum of prochloraz standard. The maximum and shape of spectra were identical with that of the prochloraz standard.

Calibration curves and mushroom samples were prepared using working standard solutions. Samples that were verified by HPLC/UV to not contain prochloraz were used as blank samples. The blank samples were spiked with 0.05; 0.5; 5.0 μg/mL of prochloraz and subjected to HPLC analysis to evaluate the accuracy and precision of the method. The slopes of the calibration curves for the samples prepared in solvent and in the matrix were compared. To minimize matrix effects, the linearity was studied using calibration curves prepared with matrix-matched standards. The limit of quantification (LOQ, 0.05 mg/kg) was determined as the lowest concentration meeting the method performance criteria for trueness and precision for a given compound. Recovery tests were repeated five times for each spiking level.

The concentration of prochloraz in extracts in μg/mL dimension using QuEChERS method is equal to the concentration of the original mushroom sample in mg/kg. 1 mL extract represents 1 g mushroom sample.

RESULTS AND DISCUSSION

Health protection of European citizens has outstanding importance in European Union whose cardinal point is the system of food control. Fungicide residues are severely controlled, for instance there is a strict limit for the application of fungicides with prochloraz active agent in mushroom cultivation. In this paper we focus on the development of novel, rapid HPLC-DAD method for the analysis of prochloraz residues that is suitable for the random analyses in the quality control of mushroom cultivation. The efficiency of the relatively cheap method was investigated thoroughly and introduced in everyday analyses in mushroom cultivation.

Application of fungicides containing prochloraz as active ingredient occurs usually on the 5th and 10th days of mushroom production by watering of casing material. Decomposition of prochloraz depends on many factors (humidity, temperature, UV-light), its half-life in water media is about 1.7 days.

For the determination of the effectiveness of the elaborated measuring method we used commercially available packed cultivated mushroom, as well as collected wild mushroom samples. For the monitoring of mushroom cultivation samples were taken from casing materials and mushrooms in first and second harvesting periods (1. harvesting period: from 17th day till 22nd day after pulling mushroom compost into the mushroom houses. 2. harvesting period: till 33rd day, that is usually the end of production.). The results of concentration of prochloraz content of wild and cultivated mushrooms and casing materials are summarized in Table 1.

Results of the measurement show that the amount of prochloraz active agent in casing material is significantly decreased for the 17. day of harvesting, which is the first day of mushroom picking. Prochloraz can be detected from...
Table 1. Prochloraz concentrations in samples (mg/kg)

| Sample                              | Prochloraz concentration (mg/kg) (±0.01) |
|-------------------------------------|------------------------------------------|
| casing material 17. day             | 5.06                                     |
| casing material 22. day             | 2.46                                     |
| casing material 33. day             | 1.01                                     |
| champignon mushroom 17. day         | 0.13                                     |
| champignon mushroom 22. day         | 0.08                                     |
| champignon mushroom 33. day         | below LOQ, close to LOD                  |
| wild champignon mushrooms           | 0.09–0.43                                |
| (5 samples originated from Hungarian local market)) |

Champignon mushroom samples harvested from this day only in 0.13 mg/kg concentration, that is significantly under the permitted exposure limit (3 mg/kg). Thus traditionally applied fungicide does not affect the product by prochloraz residues, treatment of compost by prochloraz containing fungicides on the 5th and 10th days is applicable. At the end of the first and second crop waves the amount of prochloraz decreases both in casing material and in mushroom parts. In the case of the 33rd day harvested mushrooms prochloraz concentrations were found below the quantification limit, that were very close to the detection limit of the developed HPLC-DAD method, the standard deviation is high at this concentration. We do not intend to make thorough conclusions in the case of random results of wild mushrooms, but it is interesting that the permitted exposure limit in EU for prochloraz in wild mushrooms is higher than in cultivated mushrooms. Wild mushrooms were purchased in local market thus their terroir was not known. Presumably higher values could be obtained in the case of wild mushrooms collected from the neighborhood of intensive agricultural production. Anyways the low values of the results of different wild mushroom samples are calming since the permitted exposure limit is significantly higher.

CONCLUSION

The developed measurement method is suitable for the analysis of prochloraz residues in everyday quality assurance of cultivated mushrooms. The limit of detection is 0.05 mg/kg, that is significantly lower than the permitted limit of this fungicide, thus the method was introduced in New Champignons Ltd. in everyday practice for random control analyses. Furthermore our measurement method is suitable for the quality control of compost base materials even in the case of their application for the production of organic mushroom compost due to its low detection limit (0.01 mg/kg).

The aim of our investigations was the development of a simple rapid and cheap method for the determination of prochloraz that is suitable for internal, everyday quality control. The main advantage and novelty of our approach is the use of HPLC technique that is relatively cheap and easier to access than chromatographic techniques using mass spectrometry. HPLC-DAD method was previously not described for the analysis of prochloraz in mushrooms, only HPLC-MS and HPLC-MSMS methods were elaborated.

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