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Short communication

Validation of an analytical method for 1,2,4-triazole in soil using liquid chromatography coupled to electrospray tandem mass spectrometry and monitoring of propiconazole degradation in a batch study

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ABSTRACT

1,2,4-triazole is one of the most important metabolites resulting from the degradation of a large class of pesticides, the triazole fungicides. These fungicides are widely used on fruits, vegetables and cereals. Two different analytical methods which are quick, cheap and easy to implement were developed and validated to monitor propiconazole and 1,2,4-triazole in soil using LC–MS/MS. The limits of quantification reached were 4.0 $\mu g\,kg^{-1}$ for propiconazole and 1.1 $\mu g\,kg^{-1}$ for 1,2,4-triazole. The recovery range was from 93 to 99% with a relative standard deviation <11.2% and from 83 to 97% with a RSD <7.8% for propiconazole and 1,2,4-triazole respectively. These methods: were used to monitor the degradation of propiconazole and the formation of 1,2,4-triazole in soil in a batch study lasting 28 days.

1. Introduction

The triazole class of fungicides groups together many active substances, including epoxiconazole, tebuconazole, difenoconazole, paclobutrazole and propiconazole. These are systemic fungicides used on fruits, vegetables, nuts, pulses, cereals and seed crops [1]. One of the main metabolites of these compounds is 1,2,4-triazole (1,2,4-T), with an estimated fraction of these compounds transformed into its metabolites between 3% and 44% for the triazoles class [2]. 1,2,4-T may also come from other sources such as industrial manufacturing of pharmaceuticals [3] or nitrification inhibitors [4].

In 2007, the report of the joint meeting of the FAO/WHO Panel Experts on Pesticides Residues in Food and the Environment [5] recommended the evaluation of the toxicity of 1,2,4-T because this molecule could not be linked to a specific triazole. In 2008, the experts gathered for the meeting of the same organization declared that 1,2,4-T cause reproductive toxicity and neurotoxicity [6]. During this meeting an Acceptable Daily Intake for humans (ADI) of 0.2 $mg\,kg^{-1}$ bodyweight per day and an Acute Reference Dose (ARfD) of 0.3 $mg\,kg^{-1}$ bodyweight were established. In 2011, the European Food Safety Authority (EFSA) published a Reasoned Opinion about the modification of the existing Maximum Residue Levels (MRLs) for difenoconazole; the defined toxicological reference values for 1,2,4-T were an ADI of 0.02 $mg\,kg^{-1}$ bodyweight per day and an ARfD of 0.06 $mg\,kg^{-1}$ bodyweight [7].

Propiconazole and other triazole-class fungicides are easy to extract from soil with the QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe) method [8–10] but there are few available methods for the analysis of 1,2,4-T. Moreover, 1,2,3-triazole, an isomer of 1,2,4-T, can also be found in soil [11,12]. These two molecules are difficult to separate, because they have the same weight and are both small and very polar with the same low molecular weight, making them very difficult compounds to analyse.

The EU reference laboratories for residues of pesticides [13] have developed a single residue method for the analyse of 1,2,3-triazole and 1,2,4-T using an LC–MS/MS. In this method, they use a hypercarb column which is very stable mechanically and chemically. This column is also well adapted to the analysis of polar compounds [14].

The objectives of this study were to develop an analytical method for the quantification of 1,2,4-T in soil by LC–MS and to use this method in a batch study in order to monitor the formation of 1,2,4-T and the degradation of propiconazole.

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2. Material and method

2.1. Chemicals and reagents

UPLC–MS grade methanol and acetonitrile were purchased from Biosolve (Dieuze, France). Ultrapure water (resistivity 18.2 MΩ cm) was obtained by a MilliQ ultrapure water system (EMD Millipore, USA). Certified standards of propiconazole, 1,2,3-triazole and 1,2,4-T were obtained from Sigma Aldrich (Darmstadt, Germany); these were of analytical grade (≥97%). QuEChERS salts (packaged in individual bags of 4 g $MgSO_4$, 1 g NaCl, 0.5 g sodium citrate dibasic sesquihydrate, 1 g sodium citrate tribasic dihydrate) were purchased from Biosolve (France). Stock solutions were prepared in acetonitrile for propiconazole and in methanol for 1,2,3-triazole and 1,2,4-T, and refrigerated at 4 °C for a maximum of 3 months for propiconazole solutions and for a maximum of 1 month for 1,2,3 and 1,2,4-triazole solutions. Intermediate solutions were prepared by dilution in acetonitrile for propiconazole and in pure water for 1,2,4-triazole just before use.

2.2. Sample preparation

Soil was sampled on an organic plot located in Gembloux, Belgium and managed by the CRA-W (Walloon Agricultural Research Centre). The soil is referenced as a loamy soil and its texture is 6.9% sand, 12.6% clay and 80.5% silt. Its other properties were as follow: pH in water 7.3, 17 g organic C kg^{-1} DW (dry weight), 1.6 g N – total kg^{-1} DW, 108 mg P kg^{-1} DW and 390 mg K kg^{-1} DW.

Before use, the soil was sieved to 2 mm for homogenisation and analysed to verify the absence of target compounds.

2.2.1. Propiconazole

For spike samples, sub-samples of soil (5 g) were spiked with 500 μL of propiconazole standard solutions at 0.5, 0.1 and 0.05 μg mL^{-1}, briefly manually shacked and let stand for 30 min before being extracted by the QuEChERS method as follow. 5 g of soil were placed in 50 mL plastic tube, 5 mL of ultrapure water was added and the tube was manually shaken and left to hydrate for 30 min. 10 mL of acetonitrile acidified with 1% of formic acid was added, the tube was manually shaken and left to stand for 30 min. One bag of QuEChERS salts was added. After shaking and centrifugation at 4800 rcf (relative centrifugal force) for 5 min, the supernatant was filtered on a PTFE 0.2 μm filter and analysed.

2.2.2. 1,2,4-triazole

5 g of soil were weighted into a 50 mL plastic tubes for the extraction. For spiked samples 500 μL of the appropriate 1,2,4 triazole standard solution was added and let stand for 30 min. Concentrations of the standard solutions were 0.5, 0.1 and 0.05 μg mL^{-1}. Then 10 mL of MilliQ water were added and the samples were manually stirred. Then, they were centrifuged for 5 min at 4800 rcf. The supernatant was removed and filtered through 0.2 μm PTFE filter before injection

Samples spiked with 1,2,4-T were extracted with water, manually stirred and centrifuged for 5 min at 4800 rcf before being filtered on a 0.2 μm PTFE filter and analysed immediately.

2.2.3. Matrix-matched standard solutions

For the preparation of the matrix regression curve, acetonitrile blank soil extracts were spiked with a known amount of standard solutions to obtain of 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 μg mL^{-1} as final concentration for propiconazole. Water blank soil extracts were spiked with a known amount of standard solution at the final concentrations of 0.001, 0.002, 0.005, 0.01, 0.05 μg mL^{-1} for 1,2,4-T. These matrix-matched calibration solutions samples were prepared and injected for each analysis sequence and were used as calibration curve.

2.3. Chromatographic and mass spectrometric conditions

2.3.1. 1,2,4-triazole

1,2,3-triazole and 1,2,4-triazole were analysed using an UHPLC Nexera (LC30AD, Shimadzu, USA) with an autosampler (SIL30AC, Shimadzu, USA) and a column heater (CTO20AC, Shimadzu, USA) and the separation was carried out with a hypercarb column (100 mm × 2.1 mm i.d, 3 μm particle size, Thermo Scientific, USA) set at 40 °C. 10 μL of sample was injected. The separation was achieved using three mobile phases consisting of water/methanol (97/3 v/v) as phase A, methanol as phase B and 2% formic acid in water as phase C at a flow rate of 0.25 mL min^{-1}. The gradient elution programme was: 0 min 95% A; 4 min 85.5% A and 9.5% B; 5 min 4.75% A and 90.25% B; 6.50–13 min 95% A. Phase C ran at a continuous rate of 5%. The equilibration time between each injection was 5 min.

Mass spectrometric detection was performed using a 5500 QTrap spectrometer (ABSciex, Singapore). The instrument was operated using electrospray ionisation (ESI) in positive ion mode. Source parameters were set as follows: source temperature 450 °C, ion spray voltage 5.5 kV, curtain gas 20 psi, auxiliary and nebuliser gas 50 psi. Two MRM transitions were used for both analytes: 70 > 43 for the quantification and 70 > 70 for the confirmation. No other product ion is available due the relatively small mass of the molecule. The collision exit potential and declustering potential were 10 V and 260 V respectively for the two molecules. The collision energy potential used was 10 V for the 70 > 70 transition and 27 V for the 70 > 43. All instrument settings, data acquisition and processing were controlled by the software Analyst (version 1.2.6).

2.3.2. Propiconazole

Propiconazole analyses were performed on a UHPLC–MS/MS TQD (Acquity Waters, USA) consisting of an autosampler (SNF07UPB581H), a sampler manager (M07UPA820 M) and a column heater (SNH07UPC144H) controlled by the MassLynx software (version 4.1). 2 μL of the sample was injected in a C18 column (50 mm × 2.1 mm i.d, 1.7 μm particle size, Waters Acquity UPLC® BEH C18, Waters, Ireland). The phase A elution solvent was a mix of water/methanol (90/10, v/v) and the phase B was methanol; both were acidified with 0.1% formic acid. The elution programme was: 0 min 80% A 20% B; 2.50 min 5% A 95% B; 4–5 min 80% A 20% B, at a flow rate of 0.3 mL min^{-1}. The mass spectrometer detector instrument was operated using electrospray ionisation (ESI) in positive mode. Source parameters were set as follows: source temperature 130 °C, capillary voltage 4.0 kV, cone voltage 46 V, desolvation temperature 350 °C, desolvation gas flow (nitrogen) 800 L h^{-1} and cone gas flow 80 L h^{-1}. The collision gas (argon) was run at a collision gas flow rate of 0.28 mL min^{-1}. The two chosen transitions were 342 > 69 as quantification with a collision energy of 22 eV and 342 > 159 as confirmation with a collision energy of 34 eV.

2.4. Methods validation

The methods were validated according to the EU SANTE/11945/2015 guidelines [15].

2.4.1. Selectivity and specificity

According to the guidelines, method selectivity was evaluated by the ion ratio of the specific MRM transition (70 > 70; 70 > 43) at the determined samples retention time of the calibration matrix standard.

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