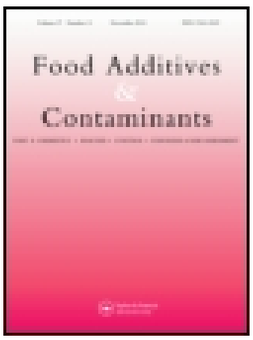

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Development and validation of an SPME-GC method for a degradation kinetics study of propiconazole I, propiconazole II and tebuconazole in blueberries in Concordia, the main production area of Argentina

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ABSTRACT

An analytical method for the simultaneous determination of propiconazole isomers and tebuconazole residues in blueberries was developed using solid-phase microextraction (SPME) coupled to gas chromatography. Confirmation was performed by gas chromatography–mass spectrometry in selected-ion monitoring mode. The SPME fibre coating selected was CWX-DVB, and the pH was adjusted to 7 with NaOH. The method is selective with adequate precision and high accuracy and sensitivity. Recoveries ranged between 97.4% and 98.9% for all compounds; and detection and quantification limits were respectively 0.21 and 0.49 μg kg^{-1} for propiconazole I; 0.16 and 0.22 μg kg^{-1} for propiconazole II; and 0.16 and 0.48 μg kg^{-1} for tebuconazole. The degradation of these fungicides in blueberries followed first-order rate kinetics. The half-life times for flowering and fruit set applications were respectively 4.0 and 10.3 days for propiconazole I, 4.0 and 11.4 days for propiconazole II, and 3.5 and 12.4 days for tebuconazole.

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Blueberries; propiconazole tebuconazole; degradation kinetics; SPME/GC-MS

Introduction

Blueberries are small fruits with high levels of nutrients beneficial to human health, such as anthocyanins and polyphenolic compounds (Lau et al. 2007; McDougall et al. 2008; Del Río et al. 2010). Fungal disease in the field is one of the main causes of economic losses in the blueberry sector (Tournas & Katsoudas 2005; Luan et al. 2007; Vásquez et al. 2007; Wharton & Schilder 2008; Rivera et al. 2009). The best control of these microorganisms is by applying good cultural practices and suitable fungicide selection. For this reason, strict controls are required to avoid their contamination during preharvesting (flowering and fruit set), harvesting and blueberry processing (Tournas & Katsoudas 2005; Luan et al. 2007; Vásquez et al. 2007; Wharton & Schilder 2008; Rivera et al. 2009; Munitz et al. 2013a). Sometimes, unsuitable agricultural practices are used during the application of these active materials. Thus, the level of pesticide residues in blueberries at harvest can be higher than the permitted level established by regulation (Grimalt & Dehouck 2016). To prevent health risks it is important to monitor and control the applications in the field, and to determine the final concentration of residues in the fruit after harvest.

Triazoles are group of systemic fungicides widely used to control several fungal pests on cereals, fruits and vegetables. They act as C-14 demethylase inhibitors (Hancock & Weete 1985; Zambonin et al. 2002). Propiconazole and tebuconazole are two triazoles used in blueberry fields located in Concordia, the main production area of Argentina (Bruzone 2010). Regulation No. 396/2005 of the European Union (EC 2005) establishes MRLs in this fruit of 1.5 mg kg^{-1} for tebuconazole and 0.05 mg kg^{-1} for propiconazole.

Numerous analytical methods for determining these pesticide residues in different products have been published. GC or LC are often used, alone or coupled to MS (Zamora et al. 2004; Walorczyk 2007, 2008; Economou et al. 2009;Šťávová et al. 2011; Walorczyk & Drożdżyński 2012; Patyal et al. 2013; Souza-Silva et al. 2013; Zhang et al. 2015; Chen et al. 2016; Pelajić et al. 2016; Rodríguez-Cabo et al. 2016). However, no work on the simultaneous

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determination of propiconazole isomers and tebuconazole in blueberries was found in the literature.

Fungicides are commonly applied at different stages of plant growth (Montes et al. 2009). Generally the initial application is during flowering, with others during fruit set. Other pesticide applications are performed after rainy days. Nowadays, there is an increasing concern about the health and safety impact of the widespread use of pesticides in agriculture. The degradation of pesticides varies from one compound to another, and it depends on different physical and chemical conditions. For these reasons, high residue concentrations could be found in the fruit as the result of the sum of applications (Montes et al. 2009; Patyal et al. 2013), and a degradation study during the different phenological stages is needed. It would be very useful to estimate the level of pesticide residues on blueberry fruits before their consumption. Some investigations have been performed on fungicide degradation in blueberry fruit post-harvest (Munitz et al. 2013b, 2014), but to our knowledge there is no work that studies different phenological stages.

The aims of the present study were the development and validation of a solid phase microextraction-gas chromatography (SPME-GC) method for the determination of propiconazole I and II and tebuconazole in blueberries, and the study of their degradation kinetics during flowering and fruit set stages.

Materials and methods

Chemicals and materials

Propiconazole I and II and tebuconazole standards of high purity (> 98%) were supplied by Sigma-Aldrich (Seelze, Germany). The stock solutions (1 g l^{-1}) were prepared by dissolving the standards in methanol HPLC grade (99.9%) purchased by Sintorgan (Buenos Aires, Argentina), and stored at –18°C in dark bottles sealed with PTFE/silicone caps. The working solutions were prepared daily by diluting with deionised water with a conductivity of 18.2 MΩs generated from an E-pure water purification system (Barnstead/Thermolyne, Bedford, MA, USA). Sodium hydroxide was purchased from Biopack (Buenos Aires, Argentina).

The SPME holder for manual use, and fibres coated with 100 µm PDMS (polydimethylsiloxane, apolar), 65 µm CWX-DVB (carbowax-divinylbenzene, polar) and 85 µm PA (polyacrylate, polar) were purchased from Supelco (Bellefonte, PA, USA). These fibre coatings were chosen for testing in order to establish the best extraction efficiency.

Chromatographic conditions

GC analyses were performed using an Agilent 6890N GC equipped with a nitrogen phosphorous detector (NPD) and an autosampler with 100-vial capacity. Separation was done using a fused silica capillary column HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium (99.999% purity) was the carrier gas at a constant flow rate of 1 ml min^{-1}. Desorption of the fibres into the injection port was carried out for 5 min in splitless mode at 250°C. The column was held at 80°C during 3.5 min after injection, then increased at a rate of 55°C min^{-1} to 280°C and maintained for 8.5 min. The detector temperature was 300°C. The different SPME fibres were conditioned by heating in the GC injection port and reused for several analyses following the manufacturer's recommendations. For confirmation analyses, an Agilent 6890N GC coupled with an Agilent 5973 MS was used, supported by reference libraries and equipped with the same column. Electron impact (EI) mass spectra were obtained at 70 eV and the system was programmed in SIM mode. The temperature of the ion source was 230°C, and MS quad temperature was 150°C.

Sampling and storage procedure

Two commercial powders were used in the experiments, which contained 25.1% propiconazole or 20% tebuconazole, under the trade names of Tilt (Syngenta Agro S.A., Argentina) and Nativo (Bayer CropScience, Argentina), respectively. Qualified personnel from the field prepared the dilutions (100 g of each powder per 100 L of solution) and applied the mixture on 850 m^2 plots that had received all routine agricultural practices except pesticide application during the 2014–15 harvest seasons. The O'Neal blueberry variety was used in this study. The actual concentrations of active ingredients in the commercial powders were confirmed with the methodology

developed in this paper (data not shown). The spraying experiments were conducted in Concordia, the main blueberry production area in Argentina. Two pesticide applications were carried out: one in the flowering stage and the other during the fruit set stage, which is common practice in blueberry fields.

Approximately 300 g of blueberries were collected randomly per sample. Approximately 15 days passed between fungicide application in the flowering stage and in the fruit set stage. For both dissipation studies, sampling to determine the residues of each fungicide was started 12 h after the application on the fruit to allow enough time for the emulsion to dry, and repeated every 5 days afterwards.

SPME procedure

Samples of 300 blueberries were pulped in a laboratory mixer. A total of 25 g of the pulp were mixed with 25 ml water and the pH was adjusted to 7.0 with sodium hydroxide, and extracted for 1 min with a vortex mixer and then the extract was centrifuged for 15 min at 4000 rpm. The supernatant was filtered with 0.45 μm filter in a vacuum manifold and the procedure was repeated, reaching a final volume of 50 ml of extract.

Pesticide extraction was done through the direct immersion mode (DI-SPME) by exposing the fibre coating for 15 min to the solution contained in an 8 ml amber glass vial. The extraction temperature was adjusted to 25°C, and magnetic stirring was performed at 200 rpm. After extraction, the fibre was introduced into the GC injector for thermal desorption.

Degradation kinetics

Plots of concentration against time were constructed for each dataset, and the equations of the best fitting curves were established via the correlation coefficients. For all samples studied, an exponential relation was found to apply (for each pesticide in both flowering and fruit set stages). The first-order kinetics was calculated from the first-order rate equation:

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t represents the concentration of pesticide at any time t; C_0 is the initial concentration (both concentrations, mg l^{-1}); and k is the rate constant (1/day).

The half-life ($t_{1/2}$) of a pesticide is a parameter used to characterise its persistence in an environmental compartment; it was determined using the equation $t_{1/2} = \ln 2/k$.

Results and discussion

SPME optimisation

The three different pHs tested (5.0, 7.0 and 9.0) did not show significant differences with any of the fibre coatings. The fibres were evaluated firstly with standard solutions of 0.1 mg kg^{-1} of each fungicide, and then with spiked blueberry samples at the same concentration to evaluate matrix effects and the performance of each polymer. In all cases, the greatest chromatographic responses were obtained with CWX-DVB (data not shown).

The CWX-DVB fibre coating and pH extraction value of 7.0 were selected for the extraction of propiconazole and tebuconazole in blueberry samples. Souza-Silva et al. (2013) determined propiconazole and tebuconazole in grapes and strawberries samples using PDMS/DVB fibre.

The extraction time was evaluated by direct immersion from 5 to 720 min to determine the adsorption equilibrium between the sample and the CWX-DVB fibre. Equilibrium was reached at 620 min, but an extraction time of only 15 min was selected to improve total analysis time, and because calibration curves were reproducible. The extraction was carried out at 0, 50, 100, 150, 200 and 250 rpm, and it was observed that the chromatographic response increased up to 200 rpm, and thus this speed was chosen.

The use of sodium chloride to improve the extraction efficiency is well known (Tsoukali et al. 2005; Fytianos et al. 2006; Chai & Tan 2009). The influence of ionic strength was evaluated by adding 10%, 20% and 30% of NaCl. The chromatographic responses for propiconazole I, propiconazole II and tebuconazole were increased with salt addition. However, the fibres were damaged when salt was added (Viñas et al. 2009), so consequently it was avoided.

Optimum desorption of analytes from the fibre was studied with the injector temperature between

230 and 260°C, and time varied in 30-s increments from 4 to 6 min. The conditions chosen were 250°C and 5 min, and no pesticide residues remained in the fibre after desorption (data not shown).

Method performance

Linearity was studied in the range of 1.0–50.0 mg kg^{-1}, and the calibration curve was constructed with five fortified samples, sequentially analysed in order of increasing concentrations. All concentrations were analysed in triplicate. Table 1 shows the parameters evaluated for the validation of the SPME method proposed.

Precision was determined by analysing samples spiked with both fungicides at all the concentrations on the same day. The RSDs obtained showed that replicates assure deviation lower than 2% for the five concentration levels studied. These values indicate that the precision of the method was satisfactory for residue control analysis (AOAC 1990).

The LOD and LOQ were established using the signal-to-noise ratio for each compound, obtained through a spiked sample at the lower concentration of the calibration curve. Ratios of 3:1 and 10:1 were used as LODs and LOQs, respectively. These values are shown in Table 1. LOD and LOQ values satisfy the MRL established by the European Union and thus the method is sufficiently sensitive (EC 2005). To our knowledge the methodology for these fungicides in blueberries did not exist prior to our study. The LODs and LOQs obtained by Chen et al. (2016) and Rodríguez-Cabo et al. (2016) in wine, by Walorczyk (2007) in wheat grains, by Walorczyk and Drożdżyński (2012) in cereals and dry animal feed, and by Souza-Silva et al. (2013) in grapes and strawberries were higher than those obtained by the present method. The LOD and LOQ ranges were 0.44–40 and 0.50–100 μg kg^{-1} for propiconazole I; 0.21–20 and 0.50–60 μg kg^{-1} for propiconazole II; and 0.16–3 and 0.55–10 μg kg^{-1} for tebuconazole, respectively.

In addition, to evaluate the accuracy of the method, a recovery study was carried out at four different concentration levels (50.0, 10.0, 2.5 and 1.0 μg kg^{-1}) in quintuplicate. The average and % RSD for each pesticide are shown in Table 1.

Pesticides were identified according to their retention times, quantification ion (target ion) and two qualifier ions in SIM mode. The retention times were 23.36, 23.48 and 23.69 min for propiconazole I, propiconazole II and tebuconazole, respectively. The quantification ions were 173 and 125 for propiconazole isomers and tebuconazole, respectively; and the qualifying ions were 69 and 259 for propiconazole isomers, and 250 and 307 for tebuconazole.

Degradation of propiconazole I, propiconazole II and tebuconazole

Results of degradation of propiconazole and tebuconazole in blueberry fruits are shown in Figures 1 and 2 for applications during flowering and fruit set, respectively. Samples were analysed in quintuplicate and the means and SDs are represented in Figures 1 and 2. In all cases studied, propiconazole I, propiconazole II and tebuconazole were found to follow first-order kinetics. The degradation parameters are summarised in Table 2. Here it can be seen that the half-life time for pesticides seems to be higher when they were applied in the fruit set stage in comparison with flowering stage. In this case, the pesticides are applied directly onto fruit of 1.5–2.0 mm diameter. However, for flowering application, the pesticides are applied on the plant without fruit, and the degradation process starts approximately 10 days before the fruit appearance. Zhang et al. (2015) studied the dissipation kinetics of propiconazole in wheat straws and soil in different fields around China, finding that the half-life times were 5.1–6.9 and 6.1–8.4 days, respectively. A study performed in apples showed that the half-life time for tebuconazole deposits ranged between 19.38 and 25.99 days with only one application and increased with a second one (Patyal et al. 2013).

Table 1. Performance characteristics of the analytical methods for blueberry samples.

Analyte	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%) ± RSD%	Precision RSD%	R^2	Equation of regression
Propiconazole I	0.21	0.49	97.4 ± 1.8	< 2%	0.9997	$y = 130.7 + 1039.6^*$
Propiconazole II	0.16	0.22	97.9 ± 1.4	< 2%	0.9999	$y = 201.3 + 1487.8^*$
Tebuconazole	0.16	0.48	98.9 ± 1.0	< 2%	0.9999	$y = -200.7 + 1291.9^*$

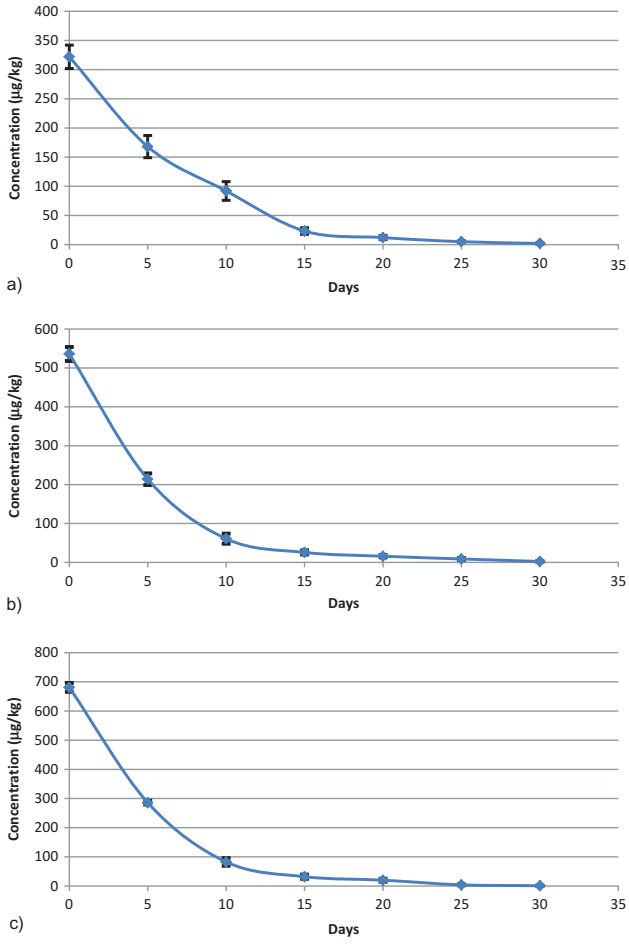

Figure 1. Degradation of fungicides applied during flowering: (a) propiconazole I; (b) propiconazole II; and (c) tebuconazole.

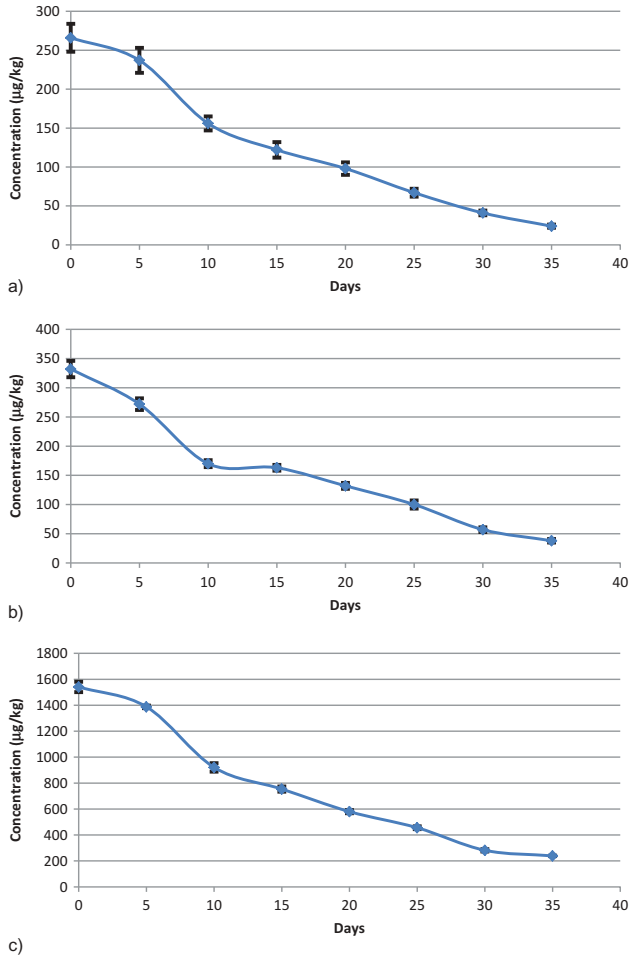

Figure 2. Degradation of fungicides applied during fruit set: (a) propiconazole I; (b) propiconazole II; and (c) tebuconazole.

The growth and maturation of blueberries finishes approximately 30–35 days after fruit set. The average final concentrations after 30 days of pesticides applied during flowering were 2.0, 2.3 and 1.0 µg kg^{-1} for propiconazole I, propiconazole II and tebuconazole, respectively. On the other hand, the final concentrations after 35 days on fruit from fruit set application were 24.0, 38.0 and 239.0 µg kg^{-1} for propiconazole I, propiconazole II and tebuconazole, respectively. The MRL for propiconazole is 50.0 µg kg^{-1}, taken as the sum of both isomers (EC 2005). The residue of this fungicide in blueberries after 35 days was higher than the MRL established by the European Union. This study was performed in a separate plot without previous applications. The sum of flowering and fruit set application residues should be considered. For this reason, good agricultural practice should be used to avoid pesticide concentrations above the MRL. Patyal et al. (2013) and Zhang et al. (2015) respectively suggested that the levels of propiconazole and tebuconazole residues mainly depend on the frequency of application.

Conclusions

Propiconazole and tebuconazole residues were determined in blueberries by employing a developed method based on DI-SPME and GC-MS analysis. The method has adequate precision and accuracy, and is selective and sensitive for the simultaneous determination of residues of both pesticides. The LOQs are lower than the MRL needed for exports to Europe. According to an extensive bibliographic research, this is the first time that the SPME methodology has been applied to the determination of propiconazole and tebuconazole in blueberries.

It was found that the degradation of propiconazole I, propiconazole II and tebuconazole in both stages of plant growth followed first-order rate

Table 2. Kinetic parameters for the degradation of propiconazole I, propiconazole II and tebuconazole applied during blueberry's flowering and fruit set stage.

Stage		Regression equation[a]	Correlation coefficient (R^2)	Rate constant (k, days^{-1})	Degradation half-life ($t_{1/2}$, days)
Flowering	Propiconazole I	$C = 5.92e^{-0.1734t}$	0.9826	0.1734	4.0
	Propiconazole II	$C = 6.11e^{-0.1726t}$	0.9718	0.1726	4.0
	Tebuconazole	$C = 6.55e^{-0.2000t}$	0.9675	0.2000	3.5
Fruit set	Propiconazole I	$C = 5.75e^{-0.0673t}$	0.9664	0.0673	10.3
	Propiconazole II	$C = 5.89e^{-0.0606t}$	0.9890	0.0606	11.4
	Tebuconazole	$C = 7.43e^{-0.0557t}$	0.9863	0.0557	12.4

Note: C = concentration (mg kg^{-1}) of the active ingredient of pesticide formulations; t = time (days).

kinetics, and an exponential model was applied. The half-life time of fungicides applied during the flowering stage was lower than that corresponding to fruit set application, and this could be attributed to degradation beginning between 10 and 15 days before blueberries fruit set. An adequate monitoring of pesticide applications in the field is important because the final residues are the sum of each application, as the time between them is not enough for total pesticide dissipation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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