Supplementary Information

Burkholderia thailandensis: the Main Bacteria Biodegrading Fipronil in Fertilized Soil with Assessment by a QuEChERS/GC-MS Method

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QuEChERS method

The extraction of fipronil and its metabolites from soil was carried out using a slightly modified QuEChERS method.[1] Into a Falcon tube of 50 mL volume, it was added 10 g of previously cleaned soil, fipronil standard solution, and 10 mL of ethyl acetate. The mixture was vortexed, and then 4 g of magnesium sulfate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were added, and vortex mixed again. The solution was then centrifuged at 3661.4 × g (6,000 rpm) for 1 min. An aliquot of 1 mL of the supernatant was collected and transferred to an Eppendorf containing 25 mg with PSA (secondary primary amine) and 150 mg of magnesium sulfate ($MgSO_4$). This mixture was vortexed and centrifuged at 3661.4 × g for 1 min. About 500 μL of the supernatant was collected and 1 μL injected into the GC-MS.

Method validation

Robustness

The parameters varied in the method for the purpose of evaluating its robustness are presented in Table S1.

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Table S1. Variations in the parameters of the QuEChERS/GC-MS method to evaluate its robustness

Parameter	Method	Variation
Ethyl acetate / mL	A = 10	a = 9.9
PSA / mg	B = 25	b = 26
Magnesium sulfate	C = 4.0 g	c = 3.8 mg
Sodium chloride / g	D = 1.0	d = 0.9
Injector temperature / °C	E = 280	e = 279
Detector temperature / °C	F = 270	f = 269
Injection volume / µL	G = 1	g = 0.95

The responses obtained in the Youden's[2] test to determine the robustness of the developed method are presented in Table S2. The retention time and peak area were evaluated.

Table S2. Responses obtained in the test of robustness with the concentration of 2.5 mg L^{-1} added to the soil (2.5 mL)

Test	Retention time / min			Peak area / A.U.		
	Fipronil	Fipronil sulfide	Fipronil sulfone	Fipronil	Fipronil sulfide	Fipronil sulfone
Method	9.815	9.639	11.548	46.289	63.803	45.487
1	9.802	9.599	11.539	47.115	64.667	46.189
2	9.882	9.606	11.632	45.108	64.567	45.173
3	9.818	9.720	11.735	45.916	65.432	44.989
4	9.811	9.682	11.444	44.030	62.998	45.512
5	9.852	9.756	11.842	45.765	63.502	46.128
6	9.713	9.633	11.443	45.123	63.103	47.007
7	9.724	9.614	11.341	46.497	64.098	46.765
8	9.816	9.658	11.538	45.577	65.120	44.564

A.U.: arbitrary units.

According to the results described in Table S3, and the statistical treatment performed by Student's *t*-test, it can be stated that the method is robust, with 95% confidence, as there is no statistically significant difference between the means of the parameters evaluated.

Table S3. Evaluation of the method robustness according to Student's *t*-test, accounting for variation in the retention time and peak area

	t calculated		*t* (0.95; 6)
	Retention time	Peak area	
Fipronil	0.068	0.092	
Fipronil sulfide	0.203	0.275	1.943
Fipronil sulfone	0.789	0.357	

Linearity

For the construction of the analytical curve of fipronil, fipronil sulfide, and fipronil sulfone, it was chosen six different concentrations and extracted according to the method QuEChERS/GC-MS. The first concentration being the LOQ of the method 62.5 ng g^{-1} and the highest point 687.5 ng g^{-1} (Table S4).

Table S4. Results obtained in the linearity analysis of the analytical method QuEChERS/GC-MS for the analyzed analytes in the range of 62.5 to 687.5 ng g^{-1}

Compound	Equation (y = ax + b)	Correlation coefficient (r)
Fipronil	y = 18.732x − 0.677	0.9923
Fipronil sulfide	y = 24.704x + 1.525	0.9907
Fipronil sulfone	y = 19.696x − 0.963	0.9912

The values are in accordance with several regulatory guides, which say that the values of the correlation coefficient should not be less than 0.99.

Limit of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) was determined by serial dilution of fipronil in soil, according to the signal-to-noise ratio where the analyte signal must be three times greater than the noise (3:1) and the limit of quantification (LOQ) using the same condition, but the signal 10 times greater than noise. The LOD and LOQ were determined by decreasing the concentrations of fipronil and its metabolites fipronil sulfide and fipronil sulfone in the range of 250 to 15.0 ng g^{-1} gradually considering the analytical signal obtained in GC-MS for each compound under study (Table S5).

Table S5. Limits of detection (LOD) and limits of quantification (LOQ) for the compounds studied in the soil

Compound	LOD / (ng g^{-1})	LOQ / (ng g^{-1})
Fipronil	15.0	65.5
Fipronil sulfide	15.0	30.0
Fipronil sulfone	15.0	65.5

The values of LOD and LOQ found when applying the QuEChERS/GC-MS method were satisfactory, for the range of work proposed for the study.

Precision

The accuracy was evaluated from the chromatographic analysis of the QuEChERS/GC-MS method, at concentrations of 125 and 625 ng g^{-1}, and each sample was analyzed in sequence. The calculation of this parameter was made from the relative standard deviations (RSD) of the results obtained for each extract (Table S6).

Table S6. Precision of the QuEChERS/GC-MS method for the compounds analyzed

Compound	RSD lowest level / %	RSD highest level / %
Fipronil	10.9	1.84
Fipronil sulfide	7.3	1.28
Fipronil sulfone	8.3	1.71

RSD: relative standard deviation.

The values are in accordance with several regulatory guides, which state that the values should not exceed 20% of the RSD.

Recovery

To evaluate the efficiency of the extraction of fipronil, fipronil sulfide, and fipronil sulfone using QuEChERS, the soil was firstly fortified in three different concentrations, each level being in triplicate. The extracts were then analyzed by GC-MS. To evaluate the recovery of the extraction method under study, three blank extracts were prepared, that is, the soil was passed without the analytes of study by any method of extraction. The extracts being fortified with fipronil and its metabolites before analyzed by GC-MS. From the results obtained in the analyses of fortified samples prior to extraction and after extraction the percentage of recovery of the method was determined, which can be seen in Table S7. Calculations were made in relation to the area of the peak related to the analyte contained in fortified sample before and after extraction by QuEChERS.

Table S7. Recovery of the QuEChERS/GC-MS method for the analyzed compounds, with the relative standard deviation (RSD) indicating the uncertainty in the experiment

Compound	Lowest level / %	Highest level / %
Fipronil	91 ± 3.7	97 ± 3.0
Fipronil sulfide	79 ± 4.2	98 ± 2.2
Fipronil sulfone	79 ± 4.5	97 ± 2.6

The values of recovery found for the two concentrations under study are in agreement with regulatory agencies, which say recovery values should be between 70 and 120%.

Repeatability

To determine the repeatability of the method for each compound, two concentrations, 62.5 and 687.5 ng g^{-1}, were used. The concentrations chosen were the highest and lowest points of the analytical curve, which are the same as those used for the precision parameter. For each concentration, seven samples were prepared, which were extracted and analyzed. This step was performed on another day, i.e., on a different day than the precision was performed.

Table S8. Repeatability of the QuEChERS/GC-MS method, for the compounds analyzed

Compound	Lowest level / %	Highest level / %
Fipronil	11.6	4.11
Fipronil sulfide	7.60	1.83
Fipronil sulfone	7.90	3.90

The repeatability values given in Table S8 are in accordance with several analytical guidelines, which state that repeatability values should be less than 15%.

References

1. Anastassiades, M.; Lehotay, S.; Stajnbaher, D.; Schenck, F. J.; *J. AOAC Int.* **2003**, *83*, 412.
2. Youden, W. J.; Steiner, E. H.; *Statistical Manual of AOAC*; Association of Official Analytical Chemistry (AOAC): Washington, 1975.