Supplementary Material for:

Assessing thiamethoxam environmental distribution in successively cultivated corn: insights from drainage lysimeter and laboratory studies

Avaliando a distribuição ambiental de tiametoxam em milho cultivado em sucessão: insights de estudos com lisímetro de drenagem e de laboratório

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In this section, you will find additional information concerning:

1. Analytical parameters employed in the thiamethoxam method detection and quantification (method validation):
    a. Linearity.
    b. Precision.
    c. Accuracy.
    d. Limit of Detection (LOD).
    e. Limit of Quantification (LOQ).
    f. Robustness.
    g. Chromatograms.
2. Additional methodological information:
    a. GOSS method and pesticide categorization.
    b. FAO soil mobility classification and some crucial parameters determined in water samples (runoff and leaching).
3. References

1. Validation of Analytical Method Parameters

a. Preparation of analytical solutions for thiamethoxam method validation

It is crucial to note that all thiamethoxam solutions were meticulously prepared using certified analytical standards (Thiamethoxam PESTANAL® 100 mg, Sigma-Aldrich; $C_8H_{10}ClN_5O_3S$; Lot #BCBT8326). Furthermore, for all preparations, ultrapure water (Puritech PT0021 – Permution®) was employed, and when needed, high-performance liquid chromatography (HPLC) grade reagents, both in liquid and solid forms, were utilized.

b. Linearity

Following ANVISA Resolution No. 899 (BRASIL, 2003), assessing linearity requires analyzing at least five distinct concentrations. Therefore, we prepared a series of ascending thiamethoxam concentrations in ultrapure water (Type I) at levels of 0.05, 0.10, 0.20, 0.50, 0.80, 1.00, 2.00, 5.00, 10.00, 20.00, 35.00, and 50.00 mg L^{-1}. These solutions were subsequently subjected to UPLC-DAD to determine the analytical response. The outcomes of this analysis are presented in Table S1 and depicted in Figure S1.

Table S1. Average absorbance values of thiamethoxam for generating the analytical reference curve.

Thiamethoxam			
Concentration (mg L^{-1})	Medium absorbance (mAU min^{-1})	Concentration (mg L^{-1})	Medium absorbance (mAU min^{-1})
0.05	0.080 ± 0.000	2.00	2.233 ± 0.009
0.10	0.139 ± 0.000	5.00	5.554 ± 0.000
0.20	0.254 ± 0.000	10.00	10.894 ± 0.038
0.50	0.567 ± 0.000	20.00	22.046 ± 0.059
0.80	0.885 ± 0.001	35.00	38.468 ± 0.005
1.00	1.229 ± 0.000	50.00	54.936 ± 0.378

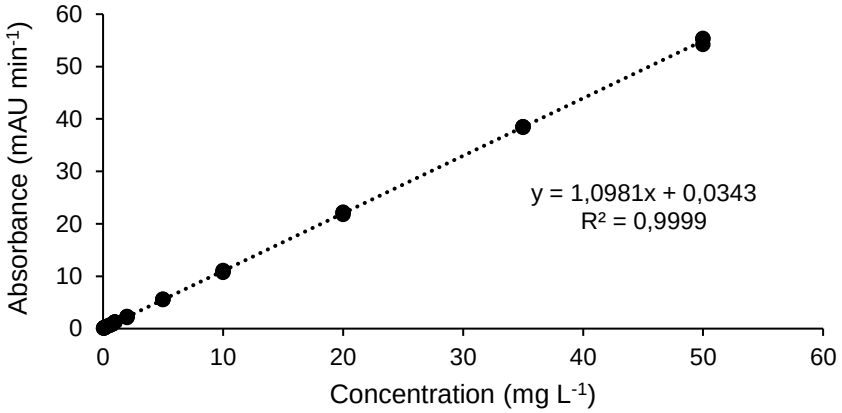

Figure S1. Thiamethoxam analytical reference curve.

According to ANVISA Resolution No. 899 (BRASIL, 2003), an R^2 (coefficient of determination) value of at least 0.99 is the minimum acceptable criterion. The results obtained using concentrations in the 0.05 to 50.00 mg L^{-1} meet this criterion satisfactorily.

c. Precision

Following Resolution No. 899 (BRASIL, 2003), concentrations of thiamethoxam were prepared (0.05, 0.10, 0.20, 0.50, 0.80, 1.00, 2.00, 5.00, 10.00, 20.00, 35.00 and 50.00 mg L^{-1}), with three repetitions for each concentration. These results are presented in Table S2 and Figures S2 and S3. The precision of an analytical method can be expressed as the relative standard deviation (RSD) or the coefficient of variation (CV%) of a series of measurements, following Equation 1. Table S2 shows the DPR value for each concentration point of the curve.

$$\text{RSD} = \frac{\text{SD}}{\text{DAC}}\ x\ 100 \qquad \text{(Eq. 1)}$$

Where SD is the standard deviation, and DAC is the average concentration.

Table S2. Intra-run and inter-run precision assays for thiamethoxam.

Intra-run precision (repeatability)				
Thiamethoxam (mg L^{-1})	Rep. 1 Absorbance (mAU min^{-1})	Rep. 2 Absorbance (mAU min^{-1})	Rep. 3 Absorbance (mAU min^{-1})	RSD (%)
0.05	0.080	0.075	0.084	5.66
0.10	0.143	0.149	0.125	8.99
0.20	0.252	0.259	0.252	1.59
0.50	0.574	0.544	0.584	3.67
0.80	0.857	0.901	0.896	2.72
1.00	1.218	1.233	1.237	0.81
2.00	2.127	2.304	2.268	4.19
5.00	5.550	5.565	5.546	0.18
10.00	11.065	10.682	10.934	1.79
20.00	22.158	22.213	21.767	1.10
35.00	38.477	38.395	38.531	0.18
50.00	55.361	54.231	55.217	1.12
Inter-run accuracy (intermediate accuracy)				
Thiamethoxam (mg L^{-1})	Rep. 1 Absorbance (mAU min^{-1})	Rep. 2 Absorbance (mAU min^{-1})	Rep. 3 Absorbance (mAU min^{-1})	DPR (%)
0.05	0.071	0.079	0.082	7.17
0.10	0.123	0.129	0.127	2.48
0.20	0.226	0.215	0.231	3.63
0.50	0.547	0.556	0.562	1.31
0.80	0.863	0.867	0.873	0.57
1.00	1.234	1.238	1.239	0.20
2.00	2.248	2.265	2.261	0.39
5.00	5.588	5.589	5.597	0.09
10.00	11.144	11.138	11.156	0.08
20.00	22.277	22.292	22.284	0.03
35.00	38.689	38.253	38.729	0.69
50.00	55.540	51.968	55.639	3.85

Note: RSD stands for relative standard deviation.

The analytical reference curves present good linearity, with R^2 values greater than or equal to 0.99, following ANVISA Resolution No. 899.

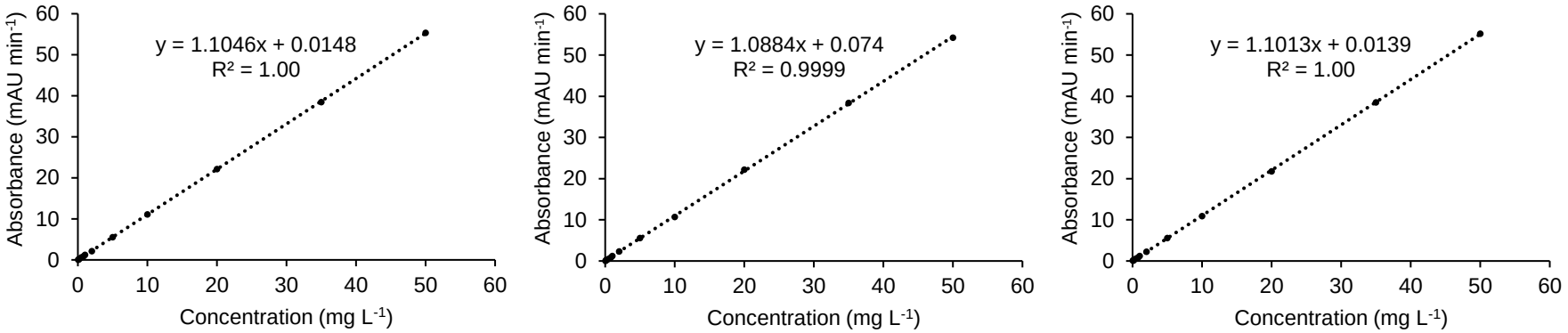

Figure S2. Intra-run precision assessment (repeatability) of thiamethoxam relative to the analytical reference curve.

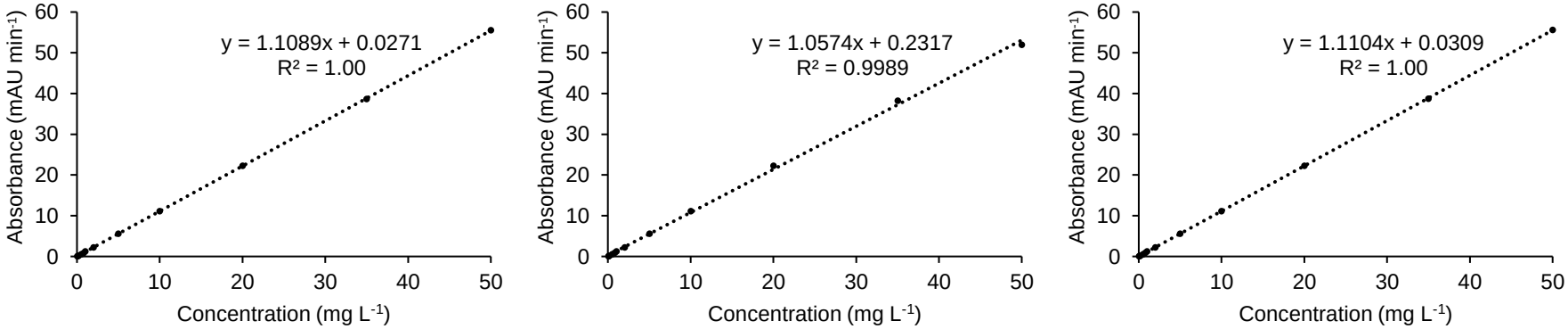

Figure S3. Inter-run precision assessment (intermediate precision) of thiamethoxam relative to the analytical reference curve.

d. Accuracy

Three analytical curves were constructed to evaluate the method's accuracy, as depicted in the accompanying graphs. The accuracy of the proposed method, specifically when applied to the analytical reference curve for thiamethoxam, yielded a value of 98.40%. Detailed theoretical concentrations and their corresponding experimental values, determined from Equation 2 concerning the regressed Y-values, are presented in Table S3.

$$Y = bx + a \quad \text{(Eq. 2)}$$

Were: Y represents the experimental concentration (mg L^{-1}); b stands for the angular coefficient obtained from the slope of the linear regression line (1.0027); x denotes the theoretical concentration (mg L^{-1}); a corresponds to the linear coefficient obtained from the intercept of the linear regression line (-0.0037).

Table S3. The theoretical and experimental concentration of thiamethoxam.

---------- Thiamethoxam ----------			
Theoretical concentration (mg L^{-1})	Experimental concentration (mg L^{-1})	Theoretical concentration (mg L^{-1})	Experimental Concentration (mg L^{-1})
0.05	0.041 ± 0.009	2.00	2.002 ± 0.002
0.10	0.095 ± 0.005	5.00	5.026 ± 0.026
0.20	0.200 ± 0.000	10.00	9.890 ± 0.110
0.50	0.485 ± 0.015	20.00	20.046 ± 0.046
0.80	0.774 ± 0.026	35.00	35.001 ± 0.001
1.00	1.088 ± 0.088	50.00	49.999 ± 0.001

e. Limit of Detection (LOD)

The limit of detection represents the minimal quantity of an analyte within a sample that can be identified under predefined experimental conditions, albeit not necessarily quantified. As per ANVISA Resolution No. 899 (BRASIL, 2003), for instrumental methods like UPLC, the LOD can be estimated by applying a threshold of three times the baseline noise or by utilizing Equation 3, as outlined below.

$$DL = \frac{SD_a \times 3.3}{SC} \quad \text{(Eq. 3)}$$

Where: SD_a is the standard deviation of the analysis of an appropriate number of blank samples; SC is the slope of the calibration curve.

Concerning the analytical reference curves for the proposed method for thiamethoxam, the LD is 0.042 mg L^{-1}.

f. Limit of Quantification (LOQ)

The limit of quantification pertains to the minimum quantity of the analyte in a sample that can be ascertained with satisfactory precision and accuracy, adhering to predefined experimental conditions. Its determination is based on Equation 4.

$$LQ = \frac{SD_a \times 10}{SC} \quad \text{(Eq. 4)}$$

Where: SDa represents the standard deviation of the analysis from a suitable number of blank samples; SC signifies the slope of the calibration curve.

Concerning the analytical reference curves for the proposed method for thiamethoxam, the LOQ is 0.141 mg L^{-1}.

g. Robustness

Variations were introduced in the mobile phase composition, flow rate, and oven temperature to assess the robustness of the proposed method. The method was modified as follows:

A) The ratio of ultrapure water to acetonitrile was adjusted to 70.5:29.5.

B) The oven temperature was increased to 24 ºC.

Following these adjustments, a triple analyte concentration was determined using a 20 mg L^{-1} sample. The results of these determinations are provided in Table 4.

Table S4. Robustness test for the proposed method for thiamethoxam.

Repetitions	Absorbance (mAU min^{-1}) of Thiamethoxam Changed Method	Absorbance (mAU min^{-1}) of Thiamethoxam Original method (without any changes)	Deviation
1	22.277	22.158	0.084
2	22.292	22.213	0.056
3	21.284	22.284	0.707
Deviation	0.578	0.063	-

It is observed that even with the change in the method in the composition of the mobile phase, in addition to the oven temperature, the deviations obtained for the concentrations are not significant. The chromatographs for thiamethoxam, obtained through the previously described UPLC-DAD method, are depicted in Figure S4(a) for the commercial product Engeo Pleno and Figure S4(b) for the analytical standards.

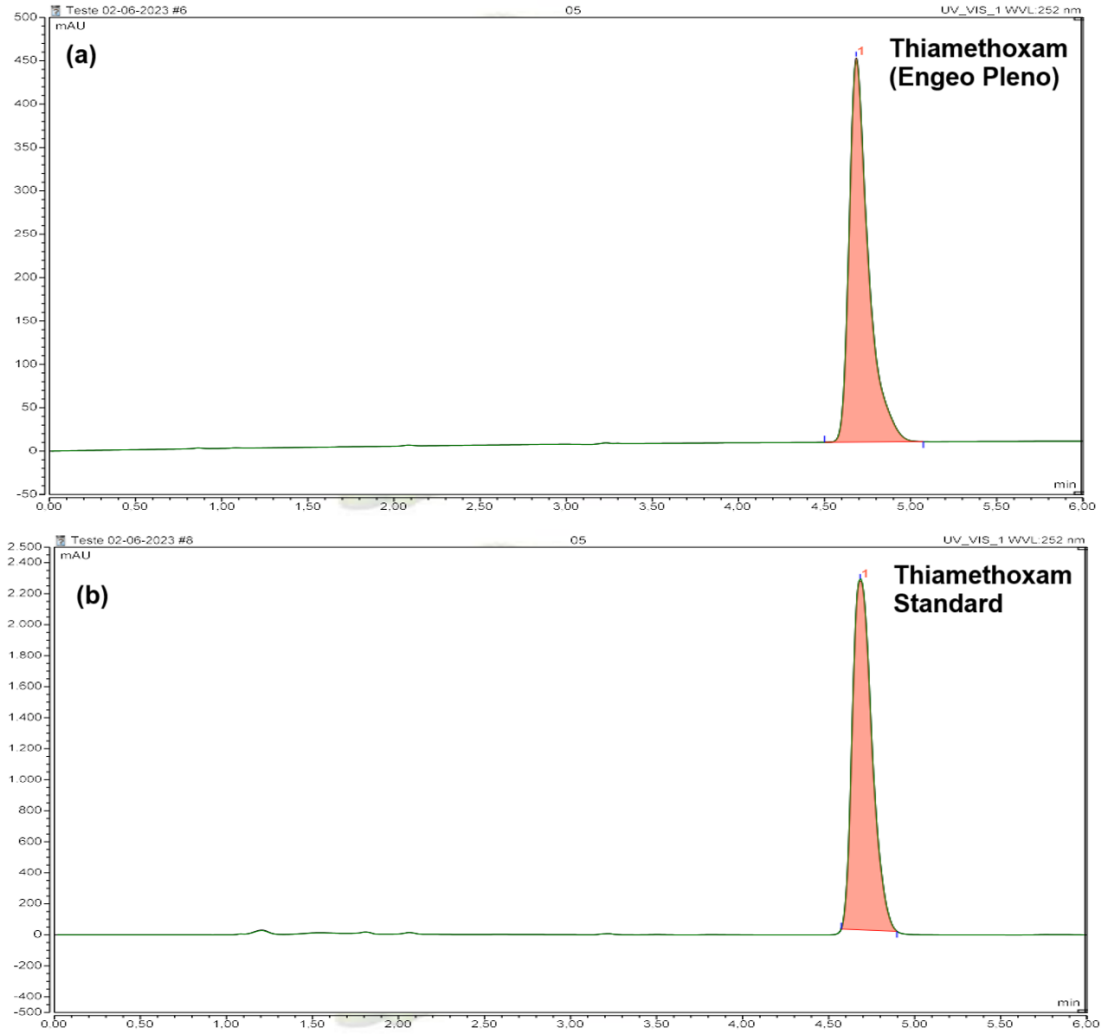

Figure S4. Chromatograms were obtained for commercial product (a) and analytical standard of thiamethoxam (b).

2. Additional methodological information

a. GOSS method and pesticide categorization

The critical parameters for interpreting GOSS include the half-life of pesticides in soil ($t_{1/2}$ soil), the soil adsorption coefficient for pesticides (K_{OC}), and the water solubility of pesticides (S_w). The ranges for these parameters are provided in Table S5.

Table S5. GOSS Index (1992) is used to assess surface water contamination risk due to pesticide transport via erosion and sediments.

Potential of pesticide transportation associated with suspended sediments				
Pesticide categories	**$t_{1/2}$ soil (days)**	**K_{OC} (L Kg^{-1})**	**S_w (µg mL^{-1})**	**Potential for contamination of surface water via pesticide transportation in suspended sediments**
(A)	≥40	≥1000	-	High potential of pesticide contamination in surface waters through runoff transportation associated with suspended sediments
(B)	≥40	≥500	≤0.5	
(C)	<1	-	-	Low potential of pesticide contamination in surface waters through runoff transportation associated with suspended sediments
(D)	≤2	≤500	-	
(E)	≤4	≤900	≥0.5	
(F)	≤40	≤500	≥0.5	
(G)	≤40	≤900	≥2	
(H)	All compounds that do not fall into the categories of low or high-potential			Moderate potential of pesticide contamination in surface waters through runoff transportation associated with suspended sediments
Potential of pesticide transportation in dissolved water				
Pesticide categories	**$t_{1/2}$ soil (days)**	**K_{OC} (L Kg^{-1})**	**S_w (µg mL^{-1})**	**Potential for contamination of surface water via pesticide transportation in dissolved water**
(I)	>35	<100000	≥1	High potential of pesticide contamination in surface waters via runoff transportation of dissolved pesticides
(J)	<35	≤700	$10 \leq Sw \leq 100$	
(K)	-	≥100000	-	Low potential of pesticide contamination in surface waters via runoff transportation of dissolved pesticides
(L)	≤1	≥1000	-	
(M)	<35	-	<0.5	
(N)	All compounds that do not fall into the categories of low or high-potential			Moderate potential of pesticide contamination in surface waters via runoff transportation of dissolved pesticides.

References: Goss (1992); Milhome et al. (2009).

b. FAO soil mobility classification and some crucial parameters determined in water samples (runoff and leaching)

Table S6 presents the soil mobility classification proposed by FAO (2000) based on the pesticide adsorption coefficient K_{OC} values. Figure S5 exhibits various parameters of water samples from runoff and leaching (Flow, Electric Conductivity – EC, Temperature, pH, total content of N, P, Ca, and Mg).

Table S6. FAO soil mobility classification criteria based on K_{OC}.

K_{OC} (mg g^{-1} or L Kg^{-1})	**Log K_{OC} (mg g^{-1} or L Kg^{-1})**	**Mobility Class**
< 10	< 1	Highly mobile
10 – 100	1 – 2	Mobile
100 – 1,000	2 - 3	Moderately mobile
1,000 – 100,000	3 – 4	Slightly mobile
10,000 – 100,000	4 – 5	Hardly mobile
> 100,000	> 5	Immobile

Reference: FAO (2000).

References:

Goss (1992). Screening Procedure for Soils and Pesticides for Potential Water Quality Impacts. Weed Technology, 6(3):701-708. https://www.jstor.org/stable/3987238

Milhome et al. (2009). Assessment of surface and groundwater potential contamination by agricultural pesticides applied in the region of Baixo Jaguaribe, CE, Brazil. Eng Sanit Ambient., 14(3):363-372. https://doi.org/10.1590/S1413-41522009000300010

Anvisa. Resolution nº 166, from July 24th, 2017. Provides for the validation of analytical methods and other provisions. Available online: http://antigo.anvisa.gov.br/documents/10181/2721567/RDC_166_2017_COMP.pdf/d5fb92b3-6c6b-4130-8670-4e3263763401. Accessed October 2023.

FAO. 2000. Appendix 2. Parameters of pesticides that influence processes in the soil. In FAO Information Division Editorial Group (Ed.), Pesticide Disposal Series 8. Assessing Soil Contamination. A Reference Manual. Rome: Food & Agriculture Organization of the United Nations (FAO). Accessed July 2009.