Revista Virtual de Química

Article

Rev. Virtual Quim. **2026**, *18(6)*, 848-860 – http://dx.doi.org/10.21577/1984-6835.20260057
©2026 Sociedade Brasileira de Química

Low-Temperature Partitioning Without Freezing in Liquid-Liquid Extraction: Determination of Thiamethoxam Residues in Percolation Water

Partição à Baixa Temperatura Sem Congelamento em Extração Líquido-Líquido: Determinação de Resíduos de Tiametoxam em Águas de Percolação

João Pedro M. Silva,[a] Anizio M. Faria[a,b,*]

[a]Universidade Federal de Uberlândia, Chemistry Institute, Separation Materials and Chromatography Research Laboratory, Zip Code 38400-902, Uberlândia-MG, Brazil
[b]Universidade Federal de Uberlândia, Pontal Institute of Exact and Natural Sciences, Separation Materials and Chromatography Research Laboratory, Zip Code 38304-402, Ituiutaba-MG, Brazil
***E-mail:** anizio@ufu.br
Submitted: June 7th, 2026 – **Accepted:** August 11th, 2026 – **Published online:** August 24th, 2026
Associate Editor Handled: João Flávio Petruci

Thiamethoxam is a neonicotinoid insecticide widely used in bean cultivation for pest control; however, its high water solubility and low soil retention increase the risk of leaching and groundwater contamination. In this study, a method based on low-temperature partitioning liquid-liquid extraction and high-performance liquid chromatography with diode-array detection was developed to determine thiamethoxam residues in leachate samples. The extraction procedure was optimized using univariate analysis to assess solvent selectivity, solvent-to-sample ratio, and freezing time. Acetonitrile was selected as the extraction solvent at a 1:1 solvent-to-sample ratio, yielding extraction recoveries of 102 ± 5 %. The main methodological innovation was eliminating the conventional freezing step used in LLE-LTP, as efficient phase separation and satisfactory thiamethoxam recovery were achieved through controlled cooling without completely freezing the aqueous phase. The method was validated in accordance with the SANTE/11312/2021 guideline and showed satisfactory selectivity, precision, accuracy, and robustness. The limit of quantification was 10 µg L^{-1}, lower than the maximum level permitted for drinking water. The method was successfully applied to leachate water samples from greenhouse bean cultivation experiments, in which thiamethoxam residues were detected after foliar spraying, indicating greater leaching potential with this application strategy.

Keywords: Neonicotinoid insecticide; green analytical chemistry; sample preparation; groundwater contamination.

1. Introduction

Thiamethoxam is a neonicotinoid insecticide widely used on crops in Brazil, especially in bean cultivation. Although it has high agronomic efficacy, its use has raised growing concerns due to adverse effects on non-target organisms, especially pollinators.[1] Evidence of acute and sublethal toxicity to bees has prompted a review of application practices, with restrictions on foliar use and a shift toward seed treatment.[2-3] In this approach, the insecticide is applied to the seed, which is then incorporated into the soil and subsequently absorbed and translocated by the plant via the vascular system.[4]

Thiamethoxam's physicochemical properties strongly shape its environmental fate. It is a highly water-soluble, polar neonicotinoid with a water solubility of approximately 4.1 g L^{-1} at 25 °C and a low octanol-water partition coefficient (log Kow ≈ −0.13).[5] Its low vapor pressure indicates negligible volatilization from water and soil. These properties favor persistence in the aqueous phase and mobility through soil, increasing the chance that it will reach surface and groundwater following irrigation or rainfall.[6-7] However, its environmental behavior is not determined solely by its high mobility, since degradation processes such as hydrolysis, photolysis, and microbial transformation also contribute to its dissipation and are strongly influenced by environmental conditions. Thus, the occurrence of thiamethoxam in aquatic systems reflects the balance between transport and degradation rather than a single persistence mechanism.[8]

From an analytical perspective, the same physicochemical characteristics that promote environmental transport can complicate the extraction and preconcentration of thiamethoxam from aqueous matrices. Its high polarity and low hydrophobicity reduce its affinity for conventional nonpolar extraction phases, and its high water solubility promotes retention in the aqueous phase.[9] Consequently,

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

efficient determination at environmentally relevant concentrations generally requires careful selection of extraction and preconcentration conditions.

The determination of thiamethoxam in environmental samples typically involves a sample preparation step, followed by instrumental analysis. Among the available techniques, liquid chromatography is the most suitable for this analyte because its low volatility and limited thermal stability make efficient gas chromatographic analysis unfeasible.[10] Despite instrumental advances, sample preparation remains the most critical step in the analytical process, often contributing the most to total analysis time, solvent consumption, and the propagation of experimental errors.[11]

In this context, there is a growing demand for sample preparation techniques that are simpler, faster, more precise, and environmentally sustainable.[12-13] Liquid-liquid extraction with low-temperature partitioning (LLE-LTP) has emerged as a promising alternative, integrating the extraction and cleanup into a single procedure.[14] In this technique, an organic solvent initially miscible with the aqueous phase is used, and phase separation is induced by lowering the temperature because cooling progressively reduces solvent miscibility, allowing phase disengagement before the aqueous phase freezes completely. In addition to simplifying the analytical workflow, LLE-LTP reduces the number of experimental steps, solvent consumption, and the risk of contamination or analyte loss. It has been successfully applied to various matrices, including environmental, food, and biological matrices.[15-21]

Despite its advantages, the cooling step — often carried out until the system (aqueous sample) is partially frozen — remains an operational bottleneck in LLE-LTP, significantly increasing sample preparation time and limiting analytical frequency.[22-24] Strategies to accelerate this process have been widely described in the literature, including the use of more powerful freezers, ultra-freezers, or cryogenics (e.g., liquid nitrogen).[25-26] However, these approaches can compromise extraction efficiency, increase analytical variability, and introduce practical limitations to cost and operational complexity. Furthermore, complete freezing may introduce additional variability associated with the freezing and subsequent phase-separation process, potentially affecting the reproducibility of analyte recovery.[14,27]

However, whether complete freezing is necessary to achieve effective phase separation and analyte recovery in LLE-LTP has received limited attention. Given these limitations, this work proposes modifying the conventional LLE-LTP procedure by inducing phase separation through controlled cooling without reaching the freezing point. A domestic refrigerator was used as a simple, accessible cooling source to assess the feasibility of achieving effective phase separation and thiamethoxam partitioning without complete freezing of the aqueous phase. The proposed modification aims to simplify sample preparation and reduce processing time while maintaining satisfactory analytical performance. Thus, the main objective of this study was to evaluate, optimize, and validate the LLE-LTP procedure under non-freezing conditions and to demonstrate its applicability to determining thiamethoxam residues in leachate water from bean cultivation.

2. Experimental

2.1. Chemicals

In this work, different solvents were used in the optimization and validation steps of the method, including chromatographic-grade acetonitrile, 99.85% (Êxodo Científica, Sumaré, Brazil), chromatographic-grade methanol, 99.5% (Êxodo Científica), ethyl acetate (Dinâmica Química, Indaiatuba, Brazil), ultrapure water (resistivity greater than 18.2 MΩ cm at 25 °C), and leachate water samples from bean cultivation. High-purity secondary standard thiamethoxam, 98.2% (Ourofino Agrociência, Uberaba, Brazil), was used as an analytical standard. An organomineral fertilizer from AgroCP (Três Pontas, Brazil) was also used, with a composition of 48% N, 28% K, and no P. For preparing the mobile phase used in the chromatographic analyses, ultrapure water and HPLC-grade methanol were each filtered through poly(tetrafluoroethylene) (PTFE) membranes with a porosity of 0.22 µm and a diameter of 45 mm.

In the experiments conducted in the greenhouse, a Red Latosol characteristic of the Brazilian Cerrado was used — whose physicochemical and textural properties are described in Table S1 of the supplementary information — as well as seeds of beans free of pesticide treatment, gently provided by the Agronomy program of the Federal Institute of Triângulo Mineiro (Ituiutaba, Brazil).

2.2. Optimization of liquid-liquid extraction with a low-temperature partitioning method

The extraction method was developed using the liquid-liquid extraction with low-temperature partitioning (LLE-LTP) technique on ultrapure water samples fortified with thiamethoxam at 10 mg L^{-1}. Briefly, 2.0 mL of the fortified water sample and 4.0 mL of extraction solvent were added to a 15 mL Falcon tube at room temperature (25 °C). The mixture was vortexed for 1 min with the tube properly sealed. The tube was then transferred to a –4 °C freezer for 4 h to promote phase separation by freezing the aqueous phase. After the freezing period, a 1.0 mL aliquot

of the liquid organic extract was collected using a syringe. The extract was transferred to an HPLC vial and analyzed by HPLC-DAD under the optimized chromatographic conditions.

Method optimization was performed through a univariate study of the main factors affecting the extraction efficiency of thiamethoxam from water samples. The evaluated parameters included extraction solvent type, sample volume, extraction solvent volume, and freezing time. For each investigated factor, the experimental condition was varied while all other parameters were kept constant. The experimental conditions evaluated for each parameter are presented in Table 1.

Table 1. Experimental conditions evaluated during optimization of the LLE-LTP method for thiamethoxam extraction from water samples

Factors	Evaluated Levels
Extraction solvent	Acetonitrile; Ethyl acetate; Methanol
Extraction solvent volume (mL)	0.5; 1.0; 2.0
Sample volume (mL)	0.5; 1.0; 2.0
Freezing time (h)	2.0; 3.0; 6.0
Cooling time (min)	15; 30; 60; 120

2.3. Method validation

The validation of the LLE-LTP/HPLC-DAD method for the determination of thiamethoxam residues in leachate water samples from bean cultivation was performed in accordance with the guidelines established in the European Commission document SANTE/11312/2021 for analytical quality control and method validation procedures for pesticide residue analysis in food and feed.[28] The evaluated validation parameters included selectivity, limit of quantification (LOQ), range, linearity, matrix effect, accuracy, precision, and robustness.

2.3.1. Selectivity

Method selectivity was evaluated by comparing chromatograms of blank leachate water samples with those of samples fortified with thiamethoxam at 10×LOQ. The absence of interfering peaks at the thiamethoxam retention time was considered indicative of adequate selectivity.

2.3.2. Limit of quantification (LOQ)

The instrumental limit of quantification (LOQ_{inst}) was experimentally determined by successive injections of standard solutions at decreasing concentrations until the lowest concentration quantifiable and repeatable chromatographic signal was obtained. The lowest concentration related to a peak area with a relative standard deviation (RSD) of less than 20% was established as the instrumental LOQ. The method LOQ was calculated considering the instrumental LOQ, the sample preparation conditions, the concentration factor, and the extraction recovery obtained by the LLE-LTP method, according to Equation 1.

$$LOQ_{met} = \frac{LOQ_{inst} \times f \times 100}{Rec(\%)} \quad (1)$$

where: LOQ_{met} = method limit of quantification, LOQ_{inst} = instrumental limit of quantification, f = concentration/dilution factor during sample preparation ($V_{sample}/V_{extractor}$), and Rec(%) = percentage recovery of thiamethoxam.

2.3.3. Range, linearity, and matrix effect

The range was established from the method LOQ up to 50×LOQ, following the recommendations of the SANTE/11312/2021 guideline.[28] Analytical curves were prepared in both solvent and blank leachate water extract at concentrations corresponding to LOQ, 2×, 5×, 10×, 15×, 20×, and 50×LOQ. The matrix-matched calibration curve was used for quantitative determination of thiamethoxam in the validation samples. The linearity of the method was evaluated by linear regression analysis of the analytical curves, correlating the analyte concentration and the chromatographic response. According to the SANTE guideline, at least five concentration levels are required for linearity evaluation. The goodness of fit was assessed using the correlation coefficient (r) and coefficient of determination (R^2). Values of ($r > 0.99$) were considered acceptable method linearity.

The matrix effect (ME) was evaluated by comparing analytical curves prepared in solvent and in blank matrix extract. Qualitative evaluation was performed by overlaying the regression lines obtained using both calibration approaches, whereas quantitative assessment was calculated by Equation 2.

$$ME(\%) = \left(\frac{S_{matrix} - S_{solvent}}{S_{solvent}} \right) \times 100 \quad (2)$$

where: S_{matrix} = slope of the analytical curve prepared in matrix extract, and $S_{solvent}$ = slope of the analytical curve prepared in solvent.

2.3.4. Accuracy and precision

Accuracy and precision were evaluated using recovery assays of leachate water samples fortified with two known concentrations of thiamethoxam. The fortified samples were subjected to the optimized LLE-LTP procedure followed by HPLC-DAD analysis. According to the SANTE/11312/2021

guideline,[28] experiments were performed in sextuplicate at two concentration levels within the range of 1 to 10×LOQ. In this study, fortification levels related to five- and ten-fold LOQ were used.

Method precision was evaluated by repeatability and within-laboratory reproducibility (intermediate precision). Repeatability was assessed by performing six extractions of fortified leachate water samples at concentrations of five- and ten-fold LOQ under the same experimental conditions, on the same day, and by the same analyst. Intermediate precision was evaluated by repeating the same procedure on two different days. Precision was expressed as relative standard deviation (RSD), calculated by Equation 3.

$$RSD = \left(\frac{s}{\bar{x}}\right) \times 100 \quad (3)$$

where: x = mean value of the thiamethoxam recovery, and s = absolute standard deviation.

Method accuracy was determined by recovery experiments using leachate water samples fortified with thiamethoxam at concentrations of five- and ten-fold LOQ. The fortified samples were subjected to the optimized LLE-LTP procedure, and the thiamethoxam recovery was calculated from the concentrations determined using the matrix-matched calibration curve, according to Equation 4.

$$\text{Recovery } (\%) = \left(\frac{[TMX]_{extracted}}{[TMX]_{spiked}}\right) \times 100 \quad (4)$$

where: $[TMX]_{spiked}$ = concentration of thiamethoxam added to the sample, $[TMX]_{extracted}$ = concentration recovered after the LLE-LTP procedure.

2.3.5. Robustness using the Youden Test

Method robustness was evaluated using a fractional factorial design 2^{7-4}, in accordance with the Youden and Steiner test,[29] which enables the simultaneous assessment of seven experimental variables through eight experiments. The robustness study was conducted using leachate water samples fortified with thiamethoxam at a concentration corresponding to 10×LOQ.

The optimized LLE-LTP method was deliberately subjected to small variations in experimental conditions in accordance with Youden's test. The evaluated factors included extraction solvent volume, sample volume, methanol brand, freezing time, mobile phase composition, flow rate, and column temperature (Table 2).

Method robustness was assessed by calculating the effect of each experimental factor on thiamethoxam recovery. The effect of each parameter was calculated using Equation 5.

$$\text{Effect}(\%) = \%\bar{R}_A - \%\bar{R}_a \quad (5)$$

where: $\%\bar{R}_A$ = mean recovery obtained under optimized conditions, $\%\bar{R}_a$ = mean recovery obtained under deliberately modified conditions.

2.4. HPLC-DAD analysis

The chromatographic analyses were performed using a Waters (Milford, MA, USA) Alliance e2695 HPLC system equipped with a diode array detector (DAD, Waters 2998 PDA). Separations were carried out on an Athena C18-WP column (150 mm × 4.6 mm i.d., 5 µm particle size, CNW Technologies). The isocratic mobile phase consisted of MeOH:water (30:70, v/v) at a flow rate of 1.0 mL min^{-1} under previously optimized conditions. Detection of thiamethoxam in the extracts was performed at 254 nm, the maximum absorption wavelength (λ_{max}). Data acquisition and processing were conducted using Empower3® software.

2.5. Analysis of percolated waters in a controlled cultivation of beans

The proposed method was applied to a real sample study using a simulated bean cultivation experiment conducted

Table 2. Matrix of experimental design for evaluating the robustness of the proposed analytical method, via Youden's test

Factors	Assays								Variations[a]	
	1	2	3	4	5	6	7	8	Optimized	Altered
Extractor volume (mL)	A	A	A	A	a	a	a	a	2.0	2.2
Sample volume (mL)	C	c	C	c	C	c	C	c	2.0	2.2
Methanol (supplier)	D	D	d	d	d	d	D	D	Êxodo	Honeywell
Freezing time (h)	B	B	b	b	B	B	b	B	2.0	2.5
Mobile phase (% MeOH)	F	f	f	F	F	f	f	F	30	32
Flow rate (mL min^{-1})	E	e	E	e	e	E	e	E	0.8	0.7
Temperature (°C)	G	g	g	G	g	G	G	g	30	32

[a] Uppercase letters (A, B, C, D, E, F, and G) represented the optimized conditions, whereas lowercase letters (a, b, c, d, e, and f) represented the deliberately modified conditions.

in a climate-controlled greenhouse (Van der Hoeven, Artur Nogueira, Brazil). Bean plants were cultivated in 5.5 L plastic pots containing 5 kg of Red Latosol, a typical soil of the Brazilian Cerrado region. A total of 84 bean plants were grown, with three plants per pot, sown at a depth of 4-5 cm and spaced 12 cm apart.

Four thiamethoxam application treatments were evaluated using seven pots per treatment. Treatment 1 consisted of a control group without thiamethoxam application. In Treatment 2 (spraying), an aqueous thiamethoxam solution was sprayed directly onto the bean leaves using a 25 mL spray bottle at approximately 20 cm, after the development of the third fully expanded trifoliate leaf (~20 days after sowing). In Treatment 3 (seed treatment), bean seeds were individually treated with 100 µL of aqueous thiamethoxam solution until absorption. In Treatment 4 (fertilizer application), an organomineral fertilizer fortified with thiamethoxam solution was applied to the soil at a depth of 3-4 cm and approximately 1 cm from the seed position. In the remaining treatments, organomineral fertilizer without thiamethoxam was used.

All treatments received the same amount of thiamethoxam, equivalent to 200 g ha^{-1}. Drip irrigation was performed daily using 0.50 L of water per pot until the 15th experimental day and 0.16 L after day 16. Leachate water samples were collected every 15 days after the 20th day of cultivation using transparent plastic bags attached to the bottom of the pots. After each collection, new clean plastic bags were installed for subsequent sampling.

3. Results and Discussion

3.1. Optimization of solvent and sample conditions for LLE-LTP

Among the parameters involved in the LLE-LTP procedure, the selection of the extraction solvent represents a critical step in method optimization, since it directly affects analyte recovery. Methanol, acetonitrile, and ethyl acetate are the most used LLE-LTP extractor solvents for pesticide extraction from solid and liquid matrices.[14,20,30] Therefore, the extraction efficiency of thiamethoxam from leachate water samples by LLE-LTP was evaluated using methanol, acetonitrile, and ethyl acetate as extraction solvents. The experiments were performed using non-optimized conditions, and the recovery results obtained for each solvent are presented in Figure 1(a).

According to Figure 1(a), methanol did not promote complete freezing of the aqueous phase within the evaluated period, making its application unsuitable under the studied conditions. This condition was also observed for Pinho *et al.*[30] In contrast, both acetonitrile and ethyl acetate enabled phase separation after freezing, allowing the collection of the liquid organic extract. However, ethyl acetate resulted in thiamethoxam recoveries below 10%, whereas acetonitrile provided recoveries close to 90%, indicating superior extraction efficiency and selectivity for the analyte. Furthermore, acetonitrile showed good compatibility with the HPLC-DAD mobile phase and was therefore selected as the extraction solvent for the proposed method.

The superior performance of acetonitrile cannot be attributed solely to its polarity or to its complete miscibility with water at room temperature. Instead, efficient extraction results from the combined effects of analyte partition equilibrium, the temperature-dependent liquid-liquid equilibrium of the water-acetonitrile system, and the physicochemical properties of thiamethoxam. Thiamethoxam is a highly polar neonicotinoid (log $K_{ow} \approx -0.3$) with high water solubility, exhibiting a low octanol-water partition coefficient.[5] Although acetonitrile is completely miscible with water at room temperature, cooling progressively reduces their mutual miscibility, promoting spontaneous

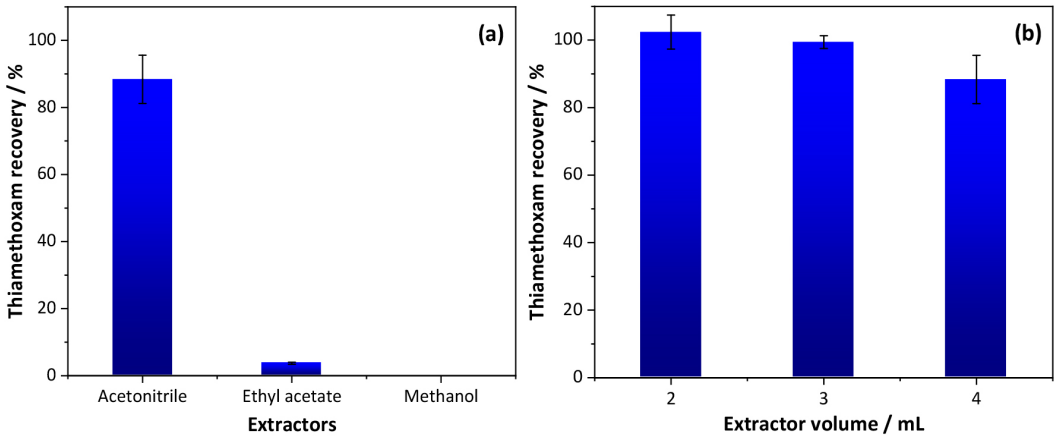


Figure 1. Recovery percentages of thiamethoxam from water samples by LLE-LTP under different extraction conditions: (a) evaluation of different extraction solvents using 2.0 mL of sample, 4.0 mL of extraction solvent, and freezing time of 4 h; (b) evaluation of different acetonitrile volumes using 2.0 mL of sample and freezing time of 4 h (n = 3)

liquid-liquid phase separation and the formation of an acetonitrile-rich phase.[27,31,32] As phase separation proceeds, the formation of an acetonitrile-rich phase provides a phase into which thiamethoxam can partition according to its distribution equilibrium. In contrast, ethyl acetate, despite being immiscible with water, provides a less favorable medium for extracting this relatively polar analyte, resulting in lower extraction efficiency. Therefore, extraction performance reflects the interplay between solvent-analyte affinity and the temperature-dependent liquid-liquid equilibrium governing phase separation, rather than solvent polarity alone.[31]

The volume of extraction solvent is an important parameter in LLE-LTP procedures, since lower solvent consumption is desirable to reduce both analytical cost and the use of toxic organic solvents, while maintaining high preconcentration capability. After selecting acetonitrile as the extraction solvent, the effect of solvent volume on thiamethoxam extraction from water samples was evaluated using 2.0, 3.0, and 4.0 mL of acetonitrile. The recovery results obtained under these conditions are shown in Figure 1(b). All evaluated volumes yielded satisfactory thiamethoxam recoveries within the 70-120 % acceptance range established by the SANTE guidelines.[28] However, the highest recovery was achieved with 2.0 mL of acetonitrile, at 102 ± 5%. Considering the analytical performance and the advantage of reduced solvent consumption, 2.0 mL was selected for the subsequent experiments.

The effect of sample volume on thiamethoxam extraction by LLE-LTP was evaluated using 0.5, 1.0, and 2.0 mL of water sample, while maintaining 2.0 mL of acetonitrile and a freezing time of 4 h. Phase separation was not satisfactorily observed for the extractions performed with 0.5 and 1.0 mL of sample, preventing recovery of the organic extract. In contrast, 2.0 mL of sample provided thiamethoxam recoveries of 102 ± 5%, and this condition was therefore selected for subsequent analyses. Moreover, the proposed method requires lower sample volumes than other LLE-LTP procedures reported for liquid matrices.[14,33,34]

3.2. Effect of freezing time on thiamethoxam extraction

LLE-LTP is an attractive sample preparation technique due to its robustness, low solvent consumption, and simple experimental procedure applied to different analytes and sample matrices with minor modifications. In addition, the freezing step promotes simultaneous phase separation and clean-up, reducing sample handling and simplifying the overall analytical procedure. However, freezing is also the most time-consuming step of the technique and may limit analytical throughput. Therefore, optimizing freezing time is essential for extraction efficiency and shorter analysis time.

The effect of freezing time on thiamethoxam extraction by LLE-LTP was evaluated using 2, 3, and 6 h under the optimized conditions of sample and extraction solvent volumes. The recovery results obtained for each freezing time are shown in Figure 2.

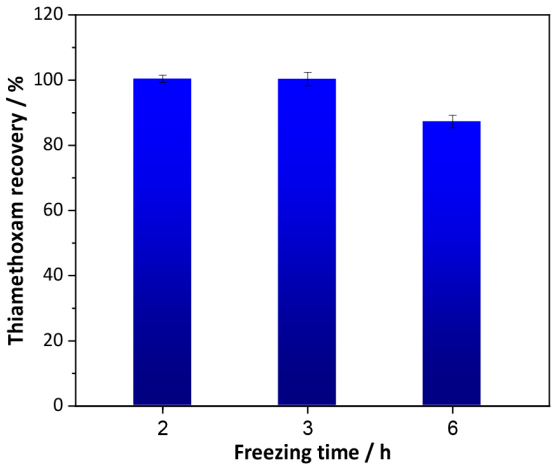


Figure 2. Percentage recovery of thiamethoxam from water samples by LLE-LTP under conditions of 2.0 mL of water samples and 2.0 mL of acetonitrile as a function of freezing time (n=3)

All evaluated conditions provided thiamethoxam recoveries within the acceptance range of 70-120% (Figure 2). Freezing times of 2 and 3 h resulted in recoveries of 100 ± 1% and 100 ± 2%, respectively, while 6 h provided a recovery of 87 ± 2%. Considering the satisfactory analytical performance and the shorter extraction time, 2 h was selected as the optimal freezing time for subsequent analyses.

During the development of this study, it was observed that cooling the extraction system to –4 °C for 2 h did not always promote complete freezing of the aqueous phase. Nevertheless, phase separation between the aqueous and organic phases was consistently observed, regardless of sample freezing, as shown in Figure 3. Since both systems exhibited similar phase separation profiles, the influence of sample freezing on thiamethoxam extraction efficiency was further investigated.

LLE-LTP under optimized conditions was applied to fortified water samples, and the organic extracts obtained from systems with and without freezing of the aqueous phase were analyzed by HPLC-DAD. The results presented in Figure 4 showed satisfactory thiamethoxam recoveries for both conditions, with values of 104 ± 6% for non-frozen samples and 99 ± 4% for frozen samples. Statistical evaluation using Student's t-test (95% confidence level, n = 3) indicated no significant difference between the recoveries. Although the statistical comparison between

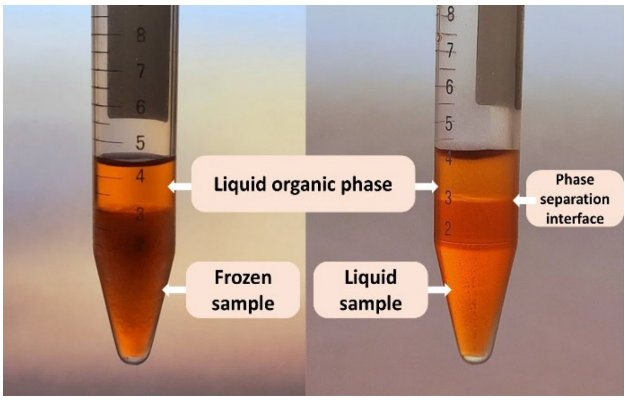


Figure 3. Phase separation in LLE-LTP systems after 2 h of cooling at –4 °C under conditions with frozen and non-frozen aqueous phases

frozen and non-frozen conditions was conducted using triplicate experiments, the suitability of the proposed procedure without complete freezing is further supported by the overall validation results. Precision and accuracy were established from six replicate analyses in accordance with the SANTE/11312/2021 guideline,[28] while robustness was demonstrated through a multivariate experimental design. Together, these independent evaluations indicate that eliminating the conventional freezing step does not adversely affect the analytical performance of the method.

The observed phase separation before complete freezing can be plausibly explained by the temperature-dependent liquid-liquid equilibrium of the water-acetonitrile system. Although water and acetonitrile are completely miscible at room temperature, cooling alters solvent-solvent interactions and shifts the thermodynamic equilibrium, progressively reducing their mutual miscibility and promoting liquid-liquid phase separation before ice formation.[27,31,32] This behavior has been previously described for aqueous acetonitrile systems and provides a physicochemical basis for cold-induced phase separation.[27,32] Under these conditions, the formation of an acetonitrile-rich phase provides a distinct phase into which thiamethoxam can partition according to its distribution equilibrium. Thus, the observed extraction can be interpreted as the result of temperature-induced phase disengagement and subsequent analyte partitioning, rather than freezing itself being the fundamental driving force for extraction. Complete freezing therefore appears to function primarily as an operational strategy traditionally used to facilitate physical phase separation.[14,27] In the proposed approach, controlled cooling alone was sufficient to promote phase disengagement and achieve satisfactory thiamethoxam recovery under the investigated conditions, enabling efficient extraction while substantially reducing sample preparation time. A detailed investigation of the underlying phase-equilibrium behavior was beyond the scope of the present study; however, the observed results are consistent with the temperature-dependent phase behavior of aqueous acetonitrile systems reported in the literature.

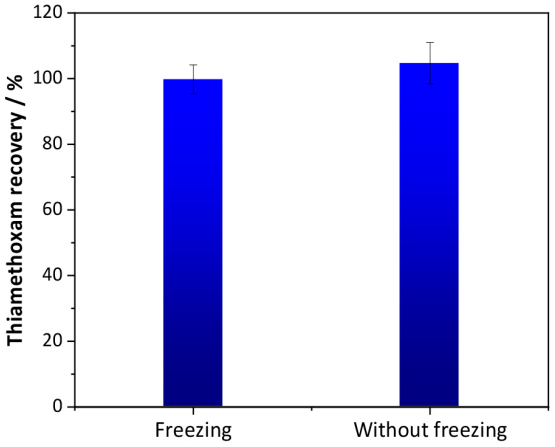


Figure 4. Recovery of thiamethoxam from fortified water samples by LLE-LTP using 2.0 mL of sample and 2.0 mL of acetonitrile after 2 h of cooling, under conditions with and without freezing of the aqueous phase (n = 3)

Based on these findings, the cooling time was further reduced to improve analytical throughput. The optimized LLE-LTP method was applied using cooling times of 15, 30, 60, and 120 min at –4 °C. The thiamethoxam recovery results are shown in Figure 5. Phase separation was observed after only 15 min of cooling, providing a thiamethoxam recovery of 86 ± 7%, which is within the acceptance criteria established by the SANTE guidelines.[28] Increasing cooling times resulted in higher recoveries and lower variability, reaching 95 ± 5% after 60 min and 99 ± 1% after 120 min.

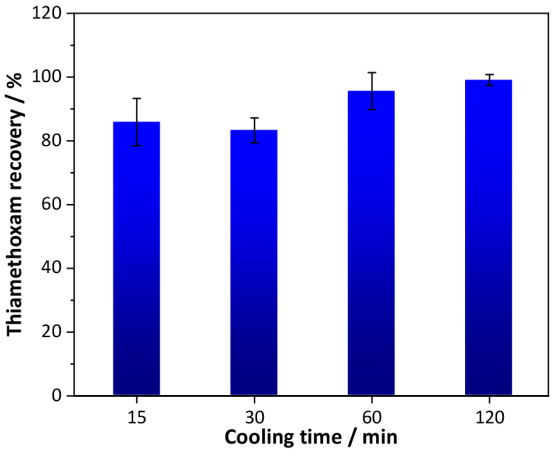


Figure 5. Effect of cooling time on the recovery of thiamethoxam from fortified water samples by LLE-LTP using 2.0 mL of sample and 2.0 mL of acetonitrile (n = 3)

These results indicate that phase separation and efficient extraction can be achieved after only 15 min of cooling, while recoveries become more stable at longer cooling

times. Therefore, 15 min of cooling was selected as the minimum cooling time that met the predefined recovery acceptance criteria and achieved satisfactory phase separation under the investigated conditions. An illustrated diagram of the optimized sample preparation procedure is presented in Figure 1S of the Supplementary Information.

3.3. Evaluation of the greenness metric of the proposed method

The sustainability of the proposed LLE-LTP method for determining thiamethoxam in water samples was evaluated using the AGREEprep metric, which assesses sample preparation against the 12 principles of Green Analytical Chemistry.[35] The metric provides a visual pictogram and an overall greenness score ranging from 0 (non-green) to 1 (ideal green procedure). Under the optimized extraction conditions (2.0 mL of sample, 2.0 mL of acetonitrile, and 15 min of cooling at −4 °C), the proposed method achieved an AGREEprep score of 0.64, indicating a relatively low environmental impact. The detailed AGREEprep assessment, including the criteria, weights, and individual scores assigned to the proposed method, is presented in Figure S2 of the Supplementary Information.

Figure 6 compares the AGREEprep evaluations of the proposed method with two representative LLE-LTP procedures previously reported for extracting neonicotinoids from water samples. Because the original publications did not report AGREEprep assessments, the scores for these methods were estimated in the present study using the experimental information available in the respective articles and following the same AGREEprep evaluation protocol used for the proposed method.

Beyond the overall greenness score, the AGREEprep pictograms indicate that the proposed method performs particularly well in the criteria related to solvent consumption, simplification of sample preparation, energy demand, and waste generation. These advantages arise from the use of only 2.0 mL of acetonitrile, the elimination of the conventional freezing step, and the absence of solvent evaporation, extract reconstitution, or additional cleanup procedures. Collectively, these characteristics reduce both reagent consumption and the number of operational steps, resulting in a more sustainable sample preparation procedure.

In contrast, the methods reported by Barros *et al.*[36] and Assis *et al.*[37] yielded lower estimated AGREEprep scores because they require larger volumes of organic solvent and include additional sample preparation steps, such as solvent evaporation and extract reconstitution, which increase energy consumption and waste generation. Therefore, although all three methods are based on the LLE-LTP principle, the proposed procedure offers a more environmentally favorable sample preparation strategy while maintaining satisfactory analytical performance for thiamethoxam determination.

3.4. LLE-LTP/HPLC-DAD method validation

After optimizing the LLE-LTP/HPLC-DAD procedure for thiamethoxam determination in leachate water samples, the analytical performance of the method was assessed in accordance with the European Commission guideline SANTE/11312/2021 for pesticide residue analysis.[28] The evaluated figures of merit included selectivity, limits of quantification, working range, linearity, matrix effect, precision, accuracy, and robustness.

Method selectivity was verified by comparing chromatograms of blank and fortified leachate water samples (10×LOQ). As shown in Figure 7, thiamethoxam was eluted at 3.87 min without coelution with matrix components, which were detected mainly between 2 and 3 min. Therefore, the method meets the selectivity criteria established by the SANTE guideline, indicating the absence of significant matrix interference in the determination.

The instrumental limit of quantification (LOQ_{inst}) was established as 0.010 mg L^{-1}, corresponding to the lowest concentration quantified with acceptable precision and accuracy. This value was confirmed by five consecutive injections, resulting in an RSD of 11.8%, in agreement with

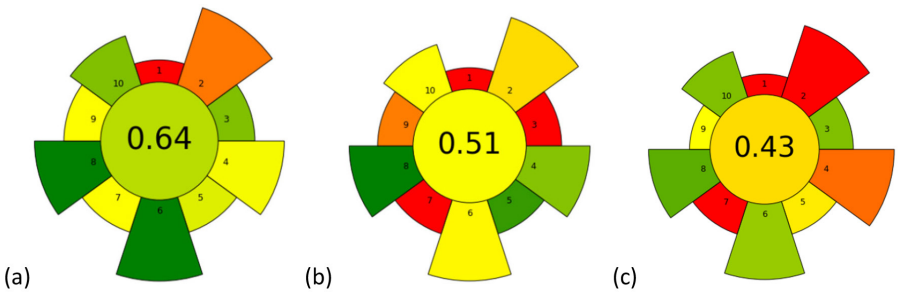


Figure 6. AGREEprep assessment of LLE-LTP methods for neonicotinoid extraction from water samples. The AGREEprep scores for the methods proposed by (b) Assis *et al.*[37] and (c) Barros *et al.*[35] were estimated in the present study based on the experimental information reported in the original publications

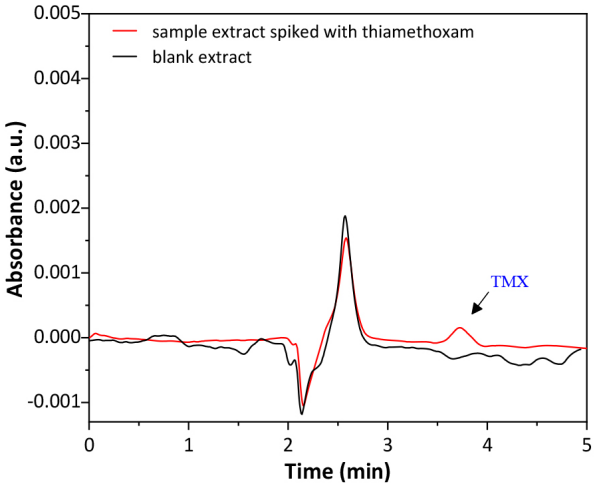


Figure 7. Overlaid chromatograms of extracts from the leachate sample blank and the sample fortified with thiamethoxam at 10×LOQ, using the optimized LLE-LTP method. Mobile phase: H_2O:MeOH (70:30, v/v), isocratic elution method with a flow rate of 0.8 mL min^{-1}; detection at 254 nm and a temperature of 30 °C

SANTE requirements. Since no concentration or dilution step was used during sample preparation and recoveries were close to 100%, the method LOQ (LOQ_{met}) was also estimated as 0.010 mg L^{-1}. This value is lower than the maximum permitted concentration for thiamethoxam in drinking water established by Brazilian legislation, 0.036 mg L^{-1},[38] demonstrating the suitability of the proposed method for monitoring trace residues in leachate waters.

Analytical curves prepared in acetonitrile and matrix extract were constructed in the range of 0.010 to 0.500 mg L^{-1} (Figure 8). Both calibration strategies showed satisfactory linearity, with correlation coefficients higher than 0.99, meeting the acceptance criteria in document SANTE/11312/2021.[28] The higher slope obtained for matrix-matched calibration indicated improved analytical sensitivity in the presence of matrix components. Comparison between the slopes of the solvent-based and matrix-matched curves revealed a matrix effect of +20.3%, classified as moderate according to the literature. Therefore, matrix-matched calibration was adopted to compensate for the signal enhancement effect and improve the quantification reliability. Although isotope-labeled internal standards can provide an additional means of compensating for matrix effects, particularly by correcting for variations during sample preparation and instrumental analysis, such standards are not always readily available for routine applications. Alternatively, the standard addition method can effectively account for matrix effects, but it requires individual calibration for each sample, increasing the analytical workload and limiting sample throughput. In this context, matrix-matched calibration was considered an appropriate and practical strategy for controlling the observed matrix effect in the present method while maintaining analytical simplicity and operational efficiency.

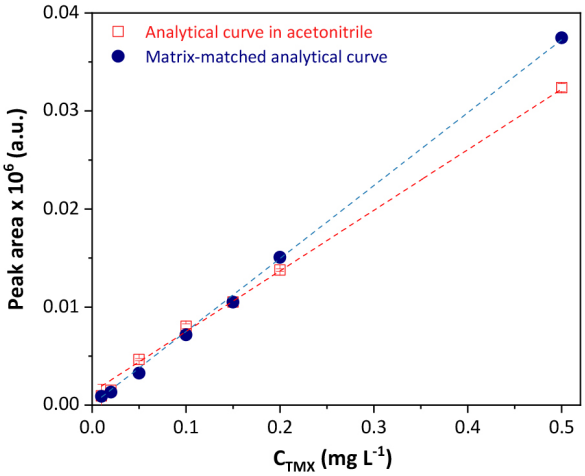


Figure 8. Analytical curves for thiamethoxam obtained by the external standard method (standard solutions of thiamethoxam prepared in acetonitrile) and the matrix-matched calibration method (fortification of the sample matrix extract with standard solutions of thiamethoxam)

The precision and accuracy of the method were evaluated at 5×LOQ and 10×LOQ levels, and the results are summarized in Table 3. Repeatability assays yielded RSD values between 4.4 and 6.7%, whereas intermediate precision showed RSD values between 10.5 and 11.2%, all below the 20% limit recommended by the validation protocol. Recovery values ranged from 93.9 to 112.9%, demonstrating adequate method accuracy for trace-level determination of thiamethoxam in leachate water samples. These results confirm the good precision and reliability of the proposed LLE-LTP/HPLC-DAD method under routine analytical conditions.

Method robustness was investigated using a Youden fractional factorial design by evaluating small deliberate variations in seven experimental parameters related to

Table 3. Precision (repeatability and intermediate precision) and recovery results obtained for the determination of thiamethoxam in leachate water samples by the LLE-LTP/HPLC-DAD method at two fortification levels

Thiamethoxam Fortification Levels (mg L^{-1})	Thiamethoxam Recovery ± RSD (%)			
	Repeatability (n = 6)		Intermediate precision (n = 12)	
	R (%)	RSD (%)	R (%)	RSD (%)
0.050 (5×LOQ)	96	7	105	11
0.100 (10×LOQ)	94	4	103	10

both sample preparation and chromatographic analysis. The results (Figure 9) showed that none of the investigated factors significantly affected thiamethoxam recovery outside the acceptable range of 70 – 120%. Among the evaluated variables, mobile phase flow rate had the greatest influence, resulting in a 13.5% decrease in recovery. This behavior is attributed to the short retention time of thiamethoxam, which increases the probability of partial overlap with matrix coextractives under higher flow conditions. Nevertheless, all variations produced deviations below 20%, confirming the robustness of the proposed method according to SANTE criteria.

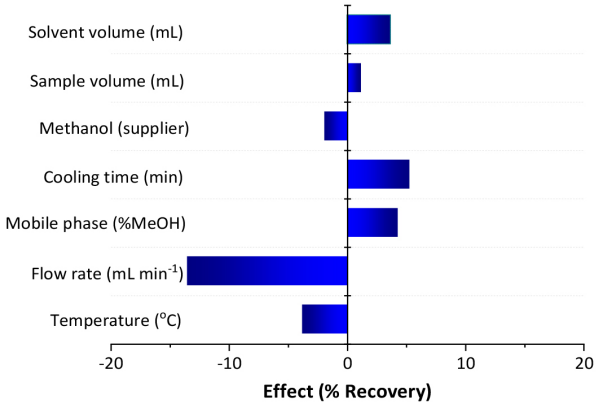


Figure 9. Effect of experimental factors on the robustness test of the thiamethoxam determination method in leachate samples using LLE-LTP and HPLC-DAD

3.5. Application of the method to leachate water samples from bean cultivation

After optimization and validation, the proposed LLE-LTP/HPLC-DAD method was applied to determine thiamethoxam residues in leachate water samples collected from a greenhouse bean cultivation experiment. Samples were collected between 5 and 30 days after pesticide application under different treatment conditions, including foliar spraying, seed treatment, and bioactive fertilizer fortified with thiamethoxam. The applied dose corresponded to 200 g ha^{-1}.

During the first 5 days after application, thiamethoxam concentrations in all treated samples were below the LOQ_{met} (< 10 µg L^{-1}). After 15 and 30 days, detectable concentrations were observed mainly for the spraying treatment, reaching 73 and 40 µg L^{-1}, respectively. In contrast, samples associated with seed treatment and fertilizer application remained below the LOQ or showed non-detectable concentrations throughout the evaluation period. These results indicate greater thiamethoxam mobility under the greenhouse conditions employed when the insecticide was applied by foliar spraying, compared with seed treatment and fertilizer application. This behavior is likely associated with greater pesticide deposition onto the soil surface during spraying, increasing its availability for dissolution and transport by irrigation water. Although these findings provide evidence of differences in leaching behavior among the application strategies evaluated, they were obtained under controlled greenhouse conditions and should not be directly extrapolated to field conditions. Nevertheless, they demonstrate the applicability of the proposed method for monitoring thiamethoxam residues in leachate water and highlight its potential for future environmental studies under real agricultural conditions.

To further contextualize the proposed method, Table 4 compares its key analytical and sample preparation characteristics with representative LLE-LTP methods previously reported for pesticide determination in water samples. The comparison covers the analytical technique, target pesticide, extraction solvent, freezing time, solvent consumption, recovery, and LOQ, enabling an assessment of the proposed procedure's operational characteristics and analytical performance relative to previously reported approaches.

As shown in Table 4, previously reported LLE-LTP methods required 1-3 h of freezing to promote phase separation, whereas the proposed procedure required only 15 min of controlled cooling at –4 °C without fully freezing the aqueous phase. In addition, the proposed method uses only 2.0 mL of acetonitrile, representing the lowest solvent

Table 4. Comparison of the proposed LLE-LTP/HPLC-DAD method with previously reported LLE-LTP methods for pesticide determination in water samples.

Sample	Analytical Techniques	Pesticides	Extraction Solvent	Freezing Time (h)	Solvent Volume (mL)	Rec (%)	LOQ (µg L^{-1})	Ref
Surface water	HPLC-DAD	Tolfenpirade	ACN	1	8.4 mL	102.4	2.0	[21]
Water	HPLC-UV	Chlorpyrifos, λ-cyhalothrin, permethrin, and bifenthrin	ACN	1	8.0 mL	> 86.0	10.0	[33]
Water	HPLC-DAD	Fluopiran	ACN	1	8.0 mL	81.5 – 113.4	6.0	[34]
Surface water	HPLC-ESI-MS/MS	Thiamethoxam	ACN	3	4.5 mL	73.4 – 78.4	~0.007	[36]
Percolated water	HPLC-DAD	Thiamethoxam	ACN	1/4	2.0 mL	94.0 – 105.0	10.0	This work

ACN: acetonitrile; Rec: recovery; LOQ: limit of quantification.

consumption among the methods compared. In contrast, the reported procedures required 4.5-8.4 mL of acetonitrile. The proposed method also provided satisfactory recovery (94.0–105.0%) and a LOQ of 10 μg L^{-1}, comparable to those reported for the HPLC-UV and HPLC-DAD methods, while the LC-ESI-MS/MS method reported by Barros *et al*.[35] achieved a substantially lower LOQ (~0.007 μg L^{-1}).

Therefore, the main advantage of the proposed method is not superior analytical sensitivity but the combination of adequate analytical performance with substantially simplified sample preparation. Eliminating the conventional freezing step reduces phase-separation time from 1-3 h to 15 min, while using only 2.0 mL of acetonitrile and avoiding additional cleanup procedures contribute to lower solvent consumption and waste generation. These characteristics make the proposed LLE-LTP/HPLC-DAD method a practical and environmentally favorable alternative for determining thiamethoxam in water samples, particularly when operational simplicity and sample throughput are prioritized.

4. Conclusions

The proposed LLE-LTP/HPLC-DAD method offers a simple, efficient approach for determining thiamethoxam residues in leachate water samples from bean cultivation. The main methodological contribution of this study was simplifying the conventional LLE-LTP procedure by demonstrating that, under the specific experimental conditions investigated, complete freezing of the aqueous phase was not necessary to achieve efficient phase separation and satisfactory analyte recovery. Controlled cooling at −4 °C for only 15 min was sufficient to promote phase separation and provide acceptable thiamethoxam recovery, substantially reducing sample preparation time. In addition, the absence of an additional cleanup step and the use of only 2.0 mL of acetonitrile reduced solvent consumption and waste generation. The method showed satisfactory selectivity, precision, accuracy, robustness, and sensitivity, in accordance with the SANTE/11312/2021 guideline. Therefore, under the conditions investigated, the proposed procedure represents a practical and environmentally favorable alternative to conventional LLE-LTP approaches, particularly when operational simplicity and sample throughput are prioritized.

Application of the method to leachate samples collected from a greenhouse bean cultivation experiment showed greater thiamethoxam mobility after foliar spraying than after seed treatment or fertilizer application. Although these observations are limited to the controlled experimental conditions evaluated and should not be directly extrapolated to field conditions, they demonstrate the applicability of the proposed method for monitoring thiamethoxam in leachate water and provide a basis for further studies under environmentally representative agricultural conditions.

Supplementary Information

Supplementary information for this article is available free of charge at https://rvq.sbq.org.br/pdf/AR2026-5205-MS.

Acknowledgements

The authors acknowledge the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (grant number APQ-01901-22) and the Financiadora de Estudos e Projetos (FINEP) (grant numbers 01.11.0135.00 and 01.13.0371.00) for financial support, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (code 001).

Author Contribution

João P. M. Silva: Methodology, Formal Analysis, Investigation, Writing – Original Draft; Anizio M. Faria: Supervision, Resources, Project Administration, Funding Acquisition, Writing – Review & Editing.

Bibliographic References

1. Paula, M. C.; Batista, N. R.; Cunha, D. A. S.; Santos, P. G.; Antonialli-Junior, W. F.; Cardoso, C. A. L.; Simionatto, E.; Impacts of the insecticide thiamethoxam on the native stingless bee *Plebeia catamarcensis* (Hymenoptera, Apidae, Meliponini). *Environmental Pollution* **2023**, *339*, 122742. [Crossref]
2. Castellanos, C. I. S.; Almeida, A. S.; Borges, C. T.; Deuner, C.; Meneghello, G. E.; Desempenho de sementes de feijão tratadas com thiamethoxam durante o armazenamento. *Agrária - Revista Brasileira de Ciências Agrárias* **2017**, *12*, 1. [Crossref]
3. Lu, C.; Hung, Y. T.; Cheng, Q.; A review of sub-lethal neonicotinoid insecticides exposure and effects on pollinators. *Current Pollution Reports* **2020**, *6*, 137. [Crossref]
4. Silva, I. H. L.; Almeida, W. A.; Souza, A. H.; Treatment of bean seeds (*Phaseolus vulgaris* L.) with systemic insecticides for the management of Cerotoma arcuata (Olivier) (Coleoptera: Chrysomelidae). *EntomoBrasilis* **2020**, *13*, e0877. [Crossref]
5. PubChem. Thiamethoxam. Available on: <https://pubchem.ncbi.nlm.nih.gov/compound/Thiamethoxam?utm_source=chatgpt.com>. Accessed on: 24th February 2026
6. Gupta, S.; Gajbhiye, V. T.; Gupta, R. K.; Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. *Bulletin of Environmental Contamination and Toxicology* **2008**, *80*, 431. [Crossref]

7. Mörtl, M.; Kereki, O.; Darvas, B.; Kláytyik, S.; Vehovszky, Á.; Gyori, J.; Székács, A.; Study on soil mobility of two neonicotinoid insecticides. *Journal of Chemistry* **2016**, *2016*, 4546584. [Crossref]
8. Hilton, M. J.; Emburey, S. N.; Edwards, P. A.; Dougan, C.; Ricketts, D. C.; The route and rate of thiamethoxam soil degradation in laboratory and outdoor incubated tests, and field studies following seed treatments or spray application. *Pest Management Science* **2019**, *75*, 63. [Crossref]
9. Kuster, M.; Alda, M. L.; Barceló, D.; Liquid chromatography-tandem mass spectrometric analysis and regulatory issues of polar pesticides in natural and treated waters. *Journal of Chromatography A* **2009**, *1216*, 520. [Crossref]
10. Jeschke, P.; Nauen, R.; In *Comprehensive Molecular Insect Science*; Gilbert, L. I., ed.; Elsevier: Amsterdam, 2005, ch. 5.3. [Crossref]
11. Raynie, D. E.; Trends in sample preparation, part I: current state of the field. *LCGC North America* **2023**, *41*, 374. [Crossref]
12. Nasiri, M.; Ahmadzadeh, H.; Amiri, A.; Sample preparation and extraction methods for pesticides in aquatic environments: A review. *TrAC Trends in Analytical Chemistry* **2020**, *123*, 115772. [Crossref]
13. Vishnu, K. N.; Aiswarya, K. N.; Sumesh, K.; In *Advances in Separation Sciences*; Ingole, P. G.; Hussain, C. M., eds.; Elsevier: Amsterdam, 2025, ch. 6. [Crossref]
14. Goulart, A. C.; Rodrigues, A. A. Z.; Heleno, F. F.; Faria, A. M.; Goulart, S. M.; Queiroz, M. E. L. R.; Liquid-liquid and solid-liquid extractions with low-temperature partitioning – A review. *Analytica Chimica Acta* **2024**, *1316*, 342795. [Crossref]
15. Santana, E. T. D.; Soares, D. F.; Faria, A. M.; Development of a methodology for the determination of pesticide residues in cajá-manga pulp (Spondias dulcis L.) using solid-liquid extraction with low-temperature partitioning. *Journal of Chemistry* **2018**, *2018*, 6012503. [Link]
16. Silva, T. L. R.; Queiroz, M. E. L. R.; Neves, A. A.; Vieira, P. A. F.; Oliveira, A. F.; Oliveira, M. G. A.; Miniaturized liquid-liquid extraction with low temperature partitioning technique (LLE/LTP) for pesticide analysis in a biological matrix by gas chromatography. *Química Nova* **2021**, *44*, 804. [Crossref]
17. Ambrosio, I. S.; Otaviano, C. M.; Castilho, L. M. B.; Santos, A. L. R.; Mendonça, J. S.; Faria, A. M.; Development and validation of a solid-liquid extraction with low-temperature partitioning method for the determination of fipronil in turtle eggshell. *Microchemical Journal* **2022**, *178*, 107393. [Crossref]
18. Araújo, F. D.; Silva, G. L. O.; Silvério, F. O.; Pinho, G. P.; Development of methods based on low-temperature partitioning (LTP) for monitoring cresols and chlorophenols in sewage sludge, soil, and water in column leaching. *Journal of Environmental Science and Health, Part B* **2023**, *58*, 530. [Crossref]
19. Costa, L. S.; Schettino, C. F.; Sicupira, L. C.; Pinho, G. P.; Silvério, F. O.; Determining pyroxasulfone herbicide in honey samples using liquid-liquid extraction with low temperature purification (LLE-LTP). *Talanta* **2024**, *273*, 125870. [Crossref]
20. Teófilo, K. R.; Sicupira, L. C.; Silvério, F. O.; Determining tolfenpyrad in water samples using liquid-liquid extraction with low temperature purification (LLE-LTP). *Journal of the Brazilian Chemical Society* **2024**, *35*, e-20240006, 1. [Crossref]
21. Assis, J. V. B.; Machado, H. G.; Rocha, Y. A.; Batista Júnior, A. C.; Nascimento, S.; Barradas, M. C.; Silva, D. M.; Chaves, A. R.; A validated LLE-LTP-LC-MS/MS workflow for pesticide biomonitoring in human plasma: Application to occupational exposure assessment in rural workers. *Environmental Chemistry and Ecotoxicology* **2026**, *8*, 1342. [Crossref]
22. Magalhães, E. J.; Nascentes, C. C.; Augusti, R.; Queiroz, M. E. L. R.; Silva, J. C. C.; Afonso, R. J. C. F.; Fast determination of benzodiazepines in human urine via liquid-liquid extraction with low temperature partitioning and LC-HRMS. *American Journal of Analytical Chemistry* **2012**, *3*, 118. [Crossref]
23. Cho, S. K.; El-Aty, A. M. A.; Park, K. H.; Park, J. H.; Assayed, M. E.; Jeong, Y. M.; Park, Y. S.; Shim, J. H.; Simple multiresidue extraction method for the determination of fungicides and plant growth regulator in bean sprouts using low temperature partitioning and tandem mass spectrometry. *Food Chemistry* **2013**, *136*, 1414. [Crossref]
24. Paula, C.; Jurisch, M.; Piccin, E.; Augusti, R.; Recognizing drug-facilitated crimes: detection and quantification of benzodiazepines in beverages using fast liquid-liquid extraction with low temperature partitioning and paper spray mass spectrometry. *Drug Testing and Analysis* **2018**, *10*, 1348. [Crossref]
25. Lopes, R. P.; Augusti, D. V.; Oliveira, A. G. M.; Oliveira, F. A. S.; Vargas, E. A.; Augusti, R.; Development and validation of a methodology to qualitatively screening veterinary drugs in porcine muscle via an innovative extraction/clean-up procedure and LC-MS/MS analysis. *Food Additives and Contaminants: Part A* **2011**, *28*, 1667. [Crossref]
26. Lopes, R. P.; Augusti, D. V.; Souza, L. F.; Santos, F. A.; Lima, J. A.; Vargas, E. A.; Augusti, R.; Development and validation (according to the 2002/657/EC regulation) of a method to quantify sulfonamides in porcine liver by fast partition at very low temperature and LC-MS/MS. *Analytical Methods* **2011**, *3*, 606. [Crossref]
27. Shao, G.; Agar, J.; Giese, R. W.; Cold-induced aqueous acetonitrile phase separation: A salt-free way to begin quick, easy, cheap, effective, rugged, safe. *Journal of Chromatography A* **2017**, *1506*, 128. [Crossref]
28. European Commission. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. Available at: <https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727>. Accessed on: 24th February 2026.
29. Youden, W. J.; Seiner, E. H.; *Statistical Manual of the AOAC - Association of the Official Analytical Chemists*. AOAC-I: Washington, 1975.

30. Pinho, G. P.; Neves, A. A.; Queiroz, M. E. L. R.; Silvério, F. O.; Optimization of the liquid–liquid extraction method and low temperature purification (LLE–LTP) for pesticide residue analysis in honey samples by gas chromatography. *Food Control*, **2010**, *21*, 1307. [Crossref]
31. Szydlowski, J.; Szykula, M.; Isotope effect on miscibility of acetonitrile and water. *Fluid Phase Equilibria* **1999**, *154*, 79. [Crossref]
32. Oence, D. N.; Gu, T.; Liquid-liquid equilibrium of the acetonitrile-water system for protein purification. *Separations Technology* **1996**, *6*, 261. [Crossref]
33. Silvério, F. O.; Silva, J. G. S.; Aguiar, M. C. S.; Cacique, A. P.; Pinho, G. P.; Análise de agrotóxicos em água usando extração líquido-líquido com partição em baixa temperatura por cromatografia líquida de alta eficiência. *Química Nova* **2012**, *35*, 2052. [Crossref]
34. Freitas, L. V. P.; Terra, G. P.; Santos, S. S.; Sicupira, L. C.; Silvério, F. O.; Optimization and validation of liquid–liquid extraction with low-temperature purification (LLE-LTP) for determining fluopyram fungicide in water samples using HPLC-DAD. *Analytical Methods* **2022**, *14*, 2945. [Crossref]
35. Pena-Pereira, F.; Tobiszewski, M.; Wojnowski, W.; Psillakis, E.; A tutorial on AGREEprep an analytical greenness metric for sample preparation. *Advances in Sample Preparation* **2022**, *3*, 100025. [Crossref]
36. Barros, A. L. C.; Abreu, C. G.; Cunha, C. C. R. F.; Rodrigues, D. A. S.; Afonso, R. J. C. F.; Silva, G. A.; Method development for simultaneous determination of polar and nonpolar pesticides in surface water by low-temperature partitioning extraction (LTPE) followed by HPLC-ESI-MS/MS. *Environmental Science and Pollution Research* **2019**, *26*, 31609. [Crossref]
37. Assis, J. V. B.; Machado, H. G.; Rocha, Y. A.; Batista Júnior, A. C.; Nascimento, S.; Barradas, M. C.; Silva, D. M.; Chaves, A. R.; A validated LLE–LTP–LC–MS/MS workflow for pesticide biomonitoring in human plasma: Application to occupational exposure assessment in rural workers. *Environmental Chemistry and Ecotoxicology* **2026**, *8*, 1342. [Crossref]
38. Ministério da Saúde. Portaria GM/MS nº 888, de 4 de maio de 2021. Available at: <https://www.saaedeitambacuri.com.br/uploads/conteudoarquivo_arquivo/2566/portariagm_msn888,de4demaiode2021douimprensanacional.pdf>. Accessed on 14th March 2024.