Multiresidue method for analysis of 240 pesticides in tomato planting soils by ultra performance liquid chromatography coupled to mass spectrometry

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ABSTRACT:

In this work, an analytical method for the determination of residues was optimized for the focus pesticides: Azoxystrobin, Boscalid, Carbendazim, Chlorantraniliprole, Clothianidin, Diafenthiuron, Difenoconazole, Dimethomorph, Spinetoram, Spinosad A, Spinosad D, Fenuron, Imidacloprid, Indoxacarb, Metalaxyl M, Methoxyfenozide, Thiamethoxan in soil derived from the tomato crop, to compare the contamination levels of these compounds in samples. The modified QuEChERS extraction method and Ultra Performance Liquid Chromatography coupled to Sequential Mass Spectrometry were used (CLUE-MS/MS), with an electronebulization ionization source in ESI (+/-) mode. The method consisted of extracting 15.0 g of soil with 15 mL of saturated calcium hydroxide solution pH 12.3 and 15 mL of acetonitrile, with consequent partitioning in a "salting out" effect using 6.0 g anhydrous magnesium sulfate and 1.5 g sodium chloride. The phases were separated by centrifugation at 3700 rpm for 7 min. Linearity between 0.2 and 20.0 µg L-1, coefficients of determination greater than 0.99. The LOQ values for the method were 13 µg kg-1 for Spinosad and 7.0 µg kg-1 for the other pesticides. The method showed good precision, with RSD values < 20%, and accuracy, with recoveries between 70 and 120% for the vast majority of the compounds analyzed. The analytical curves were prepared with reference blank soil extracts to minimize the Matrix Effect. The method was found to be suitable for the analysis of pesticide residues in the soil since it meets the validation parameters for chromatographic methods (*European Comission*, 2018). After validation, the method was used for residue analysis of these pesticides in soil samples from conventional, organic, and sustainable tomato crops. Making it possible to compare the levels of environmental impacts generated. In addition to validating the analytical method for the pesticides that were the focus of the study, validation was also achieved for another 240 compounds, including those authorized and those not authorized for use on tomato crops.

Keywords: Contaminants in Soils, Pesticide Residues, QuEChERS; UPLC-MS/MS

1. INTRODUTION

Tomato cultivation is susceptible to insect pests and diseases. Data from Carvalho's research (2017) cite that tomato cultivation is susceptible to insect pests and diseases, with the whitefly (Bemisia) being one of the main pests affecting tomatoes, *Bemisia argentifolii* and *Bemisia tabaci* being the two main species of whitefly responsible for damage to the fruit crop. In the author's work, tomato growers from the Municipality of Cambuci were interviewed (Rio de Janeiro), who report that about 60% of the growers make up to two applications of pesticides per week. These growers describe that if diseases appear or the weather is rainy, more applications are needed, up to three times a week, in about 2% of the cases.

Morphologically there is no difference between the two species. However, the former is more aggressive, since it has a higher reproduction rate and affects a larger number of host plants, and can complete its entire life cycle in the tomato plant, as well as being resistant to adverse environmental conditions and to some conventional pesticides. For this reason, it is

necessary to use various agrochemicals to control these and other pests that affect the tomato crop (ESALQ, 2017).

In this work, we chose to use the terminology oriented by the Brazilian legislation - agrochemicals- for considering that this term, although not covering, in essence, all the products used, encompasses the largest number of attributes necessary to describe the substances that make up this universe and adds more transparency and ethics for the reader, the user and the consumer of the products in which such compounds are used (SOBER, 2018).

According to the revision of the Brazilian pesticide legislation published on June 28TH of 2018 (MAPA, 2018):

> "The term pesticide is not used by any other country or international organization that deals with the subject. The Codex Alimentarius Commission, the international reference organization for food in the World Trade Organization's Agreement on the Application of Sanitary and Phytosanitary Measures (OMC), uses the English and French terms "agrotoxic" and in Spanish "plaguicida". Therefore, it is necessary to change the term pesticide to agrotoxic, in order to align Brazilian legislation with international practices.

The tomato was chosen as the object of this study because of its specific characteristics. Plantations such as tomato (S*olanum lycopersicum* L.=*Lycopersicon esculentum Mill*), require frequent attention to pest and weed infestation, which implies the need to apply pesticides with great frequency.

When they come into contact with the soil, pesticides are subject to physical-chemical processes that aggravate their action in the environment. Due to the need for rational use of agricultural inputs to minimize the environmental impacts of agriculture, many studies have been conducted to understand the behavior of these products in the soil. However, little is known about the behavior of these pesticides in tropical soils. However, little is known about the behavior of these pesticides in tropical soils. Among any form of pesticide application, the soil is the final destination of the largest quantity of these compounds (AZEVEDO, 2018).

1.1 CONTAMINATION OF SOILS BY PESTICIDES

According to Veiga's (2017) studies, even with greater control of pesticide application, the soil is the final destination of the chemicals used in agriculture, whether they are applied directly to the soil, to the aerial part of the plants, or even to the bagged fruit. When in contact with the soil, pesticides and herbicides are subject to physical-chemical processes that aggravate their action in the environment. According to the author, due to the need for rational use of

agricultural inputs to minimize the environmental impacts of agriculture, many studies have been conducted to understand the behavior of these products in the soil. However, according to the authors, little is known about the behavior of these pesticides in tropical soils. Among any form of application of pesticides, the soil is the final destination of the largest quantity of these compounds.

The soil acts as a filter, retaining many of the impurities that are dumped into it. In this way, the soil can have its quality altered by the accumulation of atmospheric pollutants, the use of pesticides and fertilizers, and solid waste, toxic and even radioactive materials. When the pollutant reaches the soil surface, it can be adsorbed, carried by wind or runoff water, or even leached by infiltration water, reaching the lower horizons and reaching the water table. Once groundwater is reached, this contaminant can be carried to other regions (CETESB, 2020).

The provision of CONAMA (2009) informs that the chemical properties of the soil, such as pH, nutrient content, ion exchange capacity, electrical conductivity, and organic matter, together with biological activities, are responsible for the adsorption, chemical fixation, oxidation, and neutralization of these pollutants.

1.2 PESTICIDES AND TOMATO CULTIVATION

The tomato is a fruit native to South America. Historical data indicate that more than 100 years ago tomatoes were already cultivated by the Incas and Aztecs in high regions of Peru and Mexico. The first countries to cultivate the product were Peru, Mexico, Bolivia, Ecuador, and Chile, according to (CURRENCY,2013). The world's largest tomato producers today are China, Brazil, United States, India, Turkey, Egypt, Italy, Iran, Spain, and Mexico, according to the FAOSTAT report (2018).

IBGE data (EPAG, 2019), show that Brazil produced 4,084,910 tons in 2018 and that in January 2019, production was 4,333,609 tons of the fruit. The regions with the highest production in 2018 were: southeast with 1,689,558 tons (São Paulo produced 811,100 tons) and the mid-west, with 1,369,014 tons (Goiás produced 1,334,500 tons).

For Junior (2019), there is no tomato plant that is resistant to most pests and diseases. This causes contamination risk for the workers involved, pesticide residues in the fruit, impacts on the environment, and increased costs.

In Brazil, ANVISA references (2018) authorize about 500 active ingredients with purposes of agricultural, domissanitary, non-agricultural, aquatic environments, and wood preservatives. Of this amount, 119 pesticides are authorized for application on tomato plantations. The same active ingredient can be marketed under the label of many formulations

and trade names, and it is common to have a mixture containing more than one active ingredient in the same product.

Carvalho (2017) records that producers cite that pesticides are used in tomato planting to combat whitefly as a priority, rickets fungus*, hollow stem, bacterial wilt, leaf miner, large fruit borer, black spot, bacterial spot, and small fruit borer. , large fruit borer, black pint, bacterial pint, and small fruit borer. According to this research, to combat such pests and diseases, several applications of pesticides are necessary. The authors mention that the farmers referred to 53 different brand-name products and an average of 12 types of pesticides per crop. Insecticides and fungicides are the products most often used by tomato growers because of the disease called rhequeima.

1.3 FATE OF AGROCHEMICALS IN THE ENVIRONMENT.

The use of agrochemicals in conventional tomato cultivation brings constant concerns due to the damage generated to the environment, especially in biotic and abiotic environments. Also, several effects are observed among field workers: weakness, nausea, dizziness, cancers, liver damage, allergies, among others. Thus, it is very important to analyze the fruit, soil, and water to quantify whether they are within the maximum residue limits (MRLs) authorized by Anvisa, according to (RIBAS and MATSUMURA, 2009).

2. METHODOLOGY - PREMISES, HYPOTHESES AND STAGES OF WORK

2.1 PREMISES

The premises of this article are:

- There is no tomato resistance to most pests and diseases
- The soil is the final destination of much of the residues from the application of pesticides in the planting methods in which they are applied;
- There is likely to be soil contamination by the application of agrochemicals in agricultural plantations that can generate environmental impacts on the biotic and abiotic environments;

2.2 HYPOTHESES

- The agrochemicals in use in Brazilian agriculture are not environmentally friendly in terms of fruit and soil contamination;
- Conventional planting with the intensive use of agrochemicals may not be environmentally friendly;

- There is a gap in agriculture, which is the absence of a validated and reliable method for determination and quantification of pesticides in soil, according to the maximum residue limits (MRL) specified by Anvisa;
- There is a gap in agriculture, which is the absence of a validated and reliable method for determination and quantification of pesticides in soil, according to the maximum residue limits (MRL) specified by Anvisa;

Knowledge of residues and contaminants in soil is important for developing actions to improve handling and control in agricultural production to reduce such contaminants.

2.3 WORK STAGES

a. Conducting a survey, with the application of an open-ended, multiple-choice questionnaire mix. This survey was applied with the growers of the areas where the samples would be collected and made it possible to survey the main pesticides used in these tomato plantations. These pesticides were identified as the "Focus pesticides" of the paper.

b. Collection of soil samples in areas of three types of cultivation: conventional, organic, and sustainable;

c. Collection of the reference blank test samples - Samples of the soils that have not been subjected to any treatment with the pesticides of interest in this work;

d. Soil characterization regarding texture, fertility and chemical composition - Stage carried out in partnership with EMBRAPA SOLOS;

e. Sample preparation for extraction - samples need to be 30 mesh (fine-grained soil), suitable for chemical extraction - this step was performed in the laboratories of EMBRAPA SOLOS (2016);

f. Analysis of the actual soil samples collected in the field.

This step was divided into:

Soil Characterization - Performed in the EMBRAPA SOLOS Laboratories;

- Determination of organic matter - Stage carried out in the EMBRAPA SOLOS Laboratories;
- - Chemical analysis for determination of soil micro-nutrients - Stage carried out in the FÉRTIMÒVEL Laboratory (EMBRAPA SOLOS);
- - Extraction, Clean-up, and Adequacy of the QuEChERS Method for Determination of Pesticide Residues in Soil Samples, through Ultra-Performance Liquid

Chromatography coupled to Sequential Mass Spectrometry - Stage carried out in the laboratories of INCQS - FIOCRUZ.

g. Calculations, statistical evaluation, and plotting of the results obtained.

2.4 AGROTOXIC SELECT TO THE STUDY

In the survey carried out with the planters, the main products used in their plantations were surveyed. Thus, it was decided to adopt the analytical method to determine residues of Abamectin, Acibenzolar-S-Methyl, Azoxystrobin, Cyromazine, Diafenthiuron, Mandipropamid, Pymetrozine, Thiamethoxan in soil from tomato cultivation (Rio de Janeiro), a major producer of tomatoes distributed among the three cropping systems studied in this work

2.5 THE EXTRACTION METHOD (QUECHERS)

The Extraction Method QuEChERS (from the English, Quick, Easy, Cheap, Effective, Rugged, and Safe) research and take to the scientific community by ANASTASSIADES *et al*. (2003), is aimed at removing the practical limitations of the previously existing multi-residue extraction methods. The method presents as main differentials, the fact that it is a fast, easy, economical, effective, robust, and safe method, as the name QuEChERS abbreviates. Diéz et al. (2006) point out that this method was developed for samples that have more than 75% water.

2.6 LITERATURE SURVEY ON THE QUECHERS METHOD COMBINED WITH CHROMATOGRAPHY FOR THE DETERMINATION FOR PESTICIDE DETERMINATION - CHRONOLOGY.

Mazzei et al., (2021) surveyed the most commonly used pesticides in tomato growing, making it possible to list the focus pesticides that guided this work.

LESUEUR et al. (2008) investigated changes for QuEChERS in the analysis of 105 pesticides by gas chromatography coupled to a mass spectrometer (CG-EM) and 46 pesticides by Ultra Performance Liquid Chromatography coupled to Sequential Mass Spectrometry (CLUE-MS/MS) after extraction by the QuECheRS method in four matrices (grape, lemon, onion, and tomato).

Drożdżyński et al. (2009) investigated 3 ecological insecticides (azadirachtin, spinosad, and rotenone) in soil, cabbage, and tomato samples using the modified QuEChERS method, with subsequent determination of the contents by CLUE-MS/MS. (2010) performed

modification of the QuEChERS method for procymidone determination in soil and leek samples complementing the work by GC-MS quantification.

RASHID et al. (2010) analyzed 19 organochlorine pesticides in soils by applying a modified QuEChERS method and clean-up consisting of liquid-liquid partitioning with n-hexane. The procedure has been validated for the determination of 19 organochlorine pesticides, hexachlorobenzene (HCB), α-HCH, β-HCH, γ-HCH, heptachlor, heptachlor epoxide (trans), aldrin, dieldrin, chlordane (trans), chlordane (cis), oxychlordane, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, p, p'-DDT, o, p'-DDT, p, p'-DDD and p, p'-DDE.

SHI et al. (2010) proposed a modified QuEChERS method for the analysis of oxadiargyl residues in soil samples, water, rice, and rice straw, with quantification by GC-ECD.

PINTO et al., (2010) researched an even more simplified version of the QuEChERS method to analyze three organo-chlorinated compounds (hexachlorobenzene; 1,2-dichlorobenzene and chlorofórm) in soil samples, followed by quantification by GC-μECD. In the work, the authors used three different types of soils: garden soil, with a high degree of organic matter; a Vertisol, with a high clay content; and certified sedimentary reference material. (clayey soil).

Martins (2010) used the QuEChERS method - determination of pesticide residues in irrigated rice crop soil, using QuEChERS modified with a saturated solution of calcium hydroxide and LC-MS/MS for the determination of residues of Clomazone, Fipronil, Imazapec, Imazetapir, Propiconazole, Tiamethoxam, and Trifloxystrobin.

RAMOS et al. (2010) developed a modified QuEChERS method for the determination of 11 pesticides in three types of soils (forestry, ornamental, and agricultural). A modified version of the QuEChERS method has been developed for the determination of organophosphorus pesticides (ethoprophos, dimethoate, diazinon, malaoxon, chlorpyrifos-methyl, fenitrion, malation, chlorpyrifos, fenamiphos, and fosmet) and a thiadiazine class pesticide (buprofezin), determining the levels by GC-NPD.

Drożdżyński et al. (2011), determined 160 pesticides in wines by employing mixed-mode dispersed solid-phase extraction and GC-MS.

COSTA (2012) conducted a study of the QuEChERS method for multi-residue determination of pesticides in peach in syrup. The LOQs of the pesticides in this study ranged from 1.0 to10.0 μg.kg-1 and were based on the curve to monitor method performance and linearity. According to the author, the analytical curves showed r values greater than 0.99; with

recovery values for drained peach in syrup ranging from 83.4 to 120.4% with RSD less than 14.9% for most analytes, and from 68.6 to 124.6% with RSD less than 19.8%.

Studies by TSIPI et al., (2015) address the quantification of residues of 2,4-D metabolites by liquid and gas chromatography coupled to mass spectrometer.

RAMOS et al. (2016) cited that the QuEChERS method was used only 8 times in the extraction of pesticides in soils and that in most cases, gas chromatography with Mass Spectrometry detection was applied (CG-EM), except in three cases, where Gas Chromatography with Electron Capture Detectors (GC-EHC); Nitrogen and Phosphorus (GC-DNF) and Micro Electron Capture Detection (GC-DμCE) were applied.

DONG et al. (2017) determined residues of metaflumizone in soil and cabbage samples by applying the QuEChERS method. The authors report that recovery values between 77.6 and 87.9% were obtained for metaflumizone in soils and cabbage, with relative standard deviation (RSD) of 3.5 and 7.9%. The method's LOD and LOQ values for the same samples were 0.001 mg.kg-1 and 0.004 mg.kg-1 respectively.

According to IGLESIAS (2016), the process of coupling Liquid Chromatography to Mass Spectrometry occurred very slowly, this was due to the incompatibility between the high flow rates used in the HPLC part that made it difficult to carry the eluent from the chromatographic column directly into the spectrometer source, which operates in high vacuum. Having solved these difficulties, Liquid Chromatography with Mass Spectrometry Coupling Interface (LC-MS) has become increasingly widespread as an excellent technique for determining residues of various analytes.

OGIHARA (2018), employed the QuEChERS method and ultra-performance liquid chromatography coupled with sequential mass spectrometry in the determination of pesticide multi-residues in soil. In their work, the three versions of the QuEChERS method, "Original", "Acetate" buffer, and "Citrate" buffer were evaluated, in the absence and in the presence of the clean up the step in the extraction of pesticides from soil and UHPLC-MS/MS with the addition of internal standard Triphenylphosphate in their quantification and confirmation.

All this research eventually led to the conclusion that classical methods for the determination of pesticides in soils are not cost-effective because these are procedures that require many steps, usually based on exhaustive matrix extraction, with subsequent clean-up steps to remove the co-extracted materials before instrumental analysis.

2.7 CHOICE OF SAMPLES FOR THE VALIDATION STEP

The samples used for the validation step were the white soil samples identified as A1BR05, which were not subjected to any pesticide treatment during planting. After the chromatographic determination, these samples were free of pesticides, not showing chromatographic signals at similar retention times as the compounds of analytical interest.

The soil used in the studies is classified as Arenic Eutrophic Hydromorphic Planosol, belonging to the mapping unit of the municipality of Tanguá, in the municipality of Rio de Janeiro. The region is flat to gently rolling with a substrate of recent alluvial sediments.

The physical-chemical properties of this soil are: pH water (1:1) = 4.8; P = 6.0 mg L-1; K = 120 mg L-1; clay = 26%; M.O. = 2.3%; Ca = 5.0 cmolc L-1; Mg = 2.0 cmolc L-1; Al = 1.7 cmolc L-1 and SMP index 5.1

2.8 REAGENTS, SOLVENTS AND GASES

Acetone P.A.; Acetonitrile - UHPLC; Anhydrous magnesium sulfate; Sodium chloride P.A.; PSA - UHPLC; Distilled water; Ultrapure water, purified in Milli-Q-Plus system; Synthetic air 99.9% pure; C18 - SPE Cartridges; Sodium chloride; Dichloromethane - Ultra Resi-Analyzed; Ethanol- UV-IR-HPLC; neutral Extran; ace argon, analytical, used as a collision gas in the CLUE-MS/MS system; Nitrogen gas, used as a desolvation gas in the electrospray source; Methanol- UV-IR-HPLC; Bondesil PSA sorbent, with a particle size of 50 μm;

2.9 EQUIPMENTS

Marconi shaker Model M227; Fanem Drying cabinet Model F 330; Vortex shaker IKA Model MS 3 Digital; Precision analytical balance; Metler Toledo; Model XP205; serial number B018030980; Precision analytical balance; SARTORIUS Model SARTORIUS - serial number 71205517; Centrifuge; Eppendorf, Model 5810R; Eppendorf Automatic Micropipettors with variable capacity; pH meter Metler Toledo S 220; Milli-Q water purification system manufactured by MilliPore; Waters Acquity Ultraperformance LC Liquid Chromatograph; Four Premier Model XE Sequential Mass Spectrometer.

2.10 METHOD OPTIMIZATION - CHROMATOGRAPHY AND MASS SPECTROMETER CONDITIONS

The analysis was performed using an Acquity UPLC® system coupled to the Quattro Premier XE® (Waters Corp., Ma, USA).

Acquity UPLC® system composed of a binary pump, autosampler and column oven.

The chromatographic separation was performed on a Waters Acquity BEH UPLC® C18 column (100 x 2,1 mm ID, 1,7 µm). Mobile phase A compositions (5 mM ammonium formate + 0.01% formic acid, pH 4.00) and mobile phase B (acetonitrile: mobile phase A, 95: 5), gradient: 0-1 min (10% B); 1 to 5.5 min (55% B); 5.5 to 10.5 min (100% B); 12 min (10% B). The flow rate used was 0.3 mL min-1, the temperature of the column oven was 30°C, the temperature of the autosampler was 25°C. The injector was set for a full-loop injection of 10 µL and the total run time was 12 min.

The Quattro Premier XE® mass spectrometer was operated with an electrospray ionization source in positive mode (ESI+). The operating parameters were set to the following conditions: capillary voltage: 3.5 kV; ion source temperature: 120 °C, desolvation temperature: 450°C; cone gas flow (N2): 20 L.h-1; desolvation gas flow (N2): 500 L.h-1; collision gas flow (Ar): 0.15 mL.min-1. The cone voltages, collision energies, and quantification and confirmation transitions for each analyte were established from a direct infusion of 1 µg. mL-1 solution. Analyte infusion was performed with mobile phases A and B (1: 1) at a flow rate of 0.1 mL.min-1 in full scan mode. After adjusting these parameters, the multiple reaction monitoring (MRM) method, used for analyte identification and quantification, was established.

The choice of mobile phase, ionization mode (positive ESI), quantification transitions, and confirmation was made according to the literature (Aguilera-Luiz et al., 2011; Rubensam *et al*., 2011) and the chemical characteristics of the analytes. Some of the parameters used in the Quattro Premier XE® system, as capillary voltage; ion source temperature; desolvation temperature, among others, were established during calibration of the instrument by the manufacturer. The precursor ions of each analyte were observed by direct infusion. In most cases, the protonated [M+ H] + ion was observed.

2.11ANALYTICAL STANDARDS

Analytical Standards of the studied pesticides and Preparation of the working solutions (fortification stock solutions)

The analytical standards of the pesticides used were purchased from AccuStandart Company. Table 1 shows the purity (%) and grade of the solid analytical standards used for the development of this work.

Chard 1 Solid analytical standards used in the work

AGROTOXIC	PURITY (%)
Azoxistrobina	99,4
Boscalida	95,5
Carbendazim	98,7
Clorantraniliprole	98,4
Clotianidina	96,5
Diafentiuron	99,9
Difenoconazol	100
Dimetomorfe	98
Espinetoram	96,8
Espinosade	96,6
Fenuron	98
Imidacloprido	99,5
Indoxacarbe	97,3
Metalaxil M	98
Metoxifenozida	99,5
Tiametoxan	100

Source: AccuStandart in New Haven, Connecticut, USA – 2018

With these standards, the fortification stock solution containing the analytes was prepared. This solution has a shelf life of only one month and must be carefully stored in an amber bottle, with bung and Teflon cap at -18°C, in ultra-cold.

All glassware used in the preparation of solutions and analyses, such as pipettes, volumetric flasks, provers, etc., were properly calibrated and identified to avoid volumetric errors in the determinations.

Initially, 10 mL of analytical stock solution 1000 mg. L-1 of each pesticide was prepared. The standards were dissolved in 0.02% methanol in glacial acetic acid, which are the same components of the mobile phase used in the liquid chromatography that will analyze the compounds, and the stock solutions were stored in amber flasks at -18 ºC.

By the method of successive dilutions, individual analytical solutions of each pesticide under study were prepared at a concentration of 100 mg. L-1, with the same solvents. From these solutions, a mixture was prepared at the concentration of 10 mg. L-1 containing all pesticides. From the standard solution 10 mg. L-1, finally, a mixture was prepared at the concentration of 0.200 mg. L-1 containing all pesticides.

Starting from the 1.0 mg L-1 intermediate mixture, analytical working solutions were prepared at concentrations of 0.4, 2.0, 4.0, 10.0, 20.0, and 40.0 µg. L-1 containing all pesticides

at each concentration for the liquid chromatograph calibration curve. For injection into the UHPLC-MS/MS system, dilutions were made in the ratio 1:1 (v/v) of these solutions in the mobile phase methanol/water, so that the final concentrations of the working solutions evaluated were 0,2; 1,0; 2,0; 5,0; 10,0, and 20,0 µg L-1 for all pesticides in the fortification stock solution. Dilutions of analytical solutions in acidified mobile phase aim to improve the ionization efficiency of the analytes, improving the chromatographic signal, peak shape, and symmetry. All solutions were stored in amber flasks and stored at -18°C.

2.12 METHOD OPTIMIZATION

The parameterization adopted for the validation of the analytical method consisted of verifying the performance. Thus, parameters such as analytical curve and linearity, the limit of detection, the limit of quantification, accuracy (recovery), and precision (repeatability and intermediate precision) have become the benchmarks for obtaining reliable results.

2.13 DETERMINATION OF THE WHITE REFERENCE SOIL

Due to the complexity of the matrix and the low concentration levels at which pesticides are found in soil (order of ppm to ppb), sample preparation was critical to obtain reliable results.

The most difficult step was to get a white soil sample, this is a pesticide-free product that could serve as a zero reference for the studies. It was this soil that was intended to be contaminated with the pesticides to follow the optimization using the QuEChERS method.

For the white soil check, the soil sample coded as A1BR05 was used in two treatments:

2.12.1 Treatment 01

In five 50 mL Falcon centrifuge tubes, 15 g of the soil was weighed and treatment 01 was carried out, based on the original QuEChERS Method: 15g soil + 5 mL H2O; vortex 30 sec, 1 mL Surrogate (Propoxur 1.0 µg/mL); vortex 30 sec; 15 min hold; 15 mL ACN grade UHPLC; Vortex 30 sec; 6g MgSO4 + 1.5g NaCl; Centrifugation (7 min); extraction of the supernatant and dilution with methanol RP 1:1 for injection into the liquid chromatograph.

The Propoxur 1.0 µg. mL-1 solution (Surrogate) was used as a marker. If the chromatogram of the blank appeared without peaks, it was necessary to ensure that the system manifested sensitivity to the compounds, and propoxur was the compound that brought this certainty.

2.12.2 Treatment 02

A modified method treatment with Calcium Hydroxide solution at pH = 12.6 was also tested to obtain a better background of the samples for the soil matrix as follows:

In five 50 mL Falcon centrifuge tubes 15g of soil were weighed and treatment 02 - 15g soil + 5 mL H2O; vortex 30 sec; 1 mL Surrogate; vortex 30 sec; 5 mL Ca(OH)2 Solution pH 12.6; 5 min hold; 15 mL ACN grade UHPLC; vortex 30 sec; 6g MgSO4 + 1.5g NaCl; centrifugation (7 min); extraction of the supernatant and dilution with methanol RP 1;1 for injection into the liquid chromatograph.

The samples of soil A1BR05 were free of pesticides in both treatments. From this stage on, sample A1BR05 became the reference soil of this study.

The previous two treatments were employed to the soil samples spiked with the fortification solution containing the interested analytes. The method was initially optimized for extraction of the focus pesticides: Azoxystrobin, Boscalid, Carbendazim, Chlorantraniliprole, Clothianidin, Diafenthiuron, Difenoconazole, Dimethomorph, Spinetoram, Spinosad A, Spinosad D, Fenuron, Imidacloprid, Indoxacarb, Metalaxyl M, Methoxyfenozide, Thiametoxan from tomato crop soil samples, followed by determination by UHPLC-MS/MS (Ultra Performance Liquid Chromatography), which requires the array to be clean, minimizing background interferences as much as possible (matrix effect - background). Thus, treatments 1 and 2 were the starting points for the extraction of these pesticides from the soil matrix.

In both treatments, the extracts were quite clear. Even so, fractions from each of the aforementioned assays were tested in a dispersive clean-up step. In this step, a dispersive solid-phase extraction of the PES cleanup was tested, generating 4 more treatments, totaling 8 different assays.

The extract was filtered through a PTFE membrane and then, 1 mL of extract was transferred to a volumetric flask, dissolved with 1 mL of Methanol, and this final solution was transferred to a chromatographic vial. From this point on, 5 microliters of each sample were injected in duplicate into the Ultra Performance Liquid Chromatograph coupled to a mass spectrometer. The tests were done in duplicate and the results are plotted in chart 2

Chart 2 Results of treatments 1 and 2 of the analysis of the focus pesticides by ultra-performance liquid chromatography

Active Ingredient	Recovery Factor without clean-up (%)		Recovery Factor Post Clean-up (%)	
	Treatment 01	Treatment 02	Treatment 01	Treatment 02
Abamectina	88/115	65/70	97,5	115
Diafentiuron	43/37	67/72	53	81,2
Azoxistrobina	101/100	93/94	162,5	160
Pimetrozina	30/28	81/75	30	120
Acibenzolar-S-Metílico	138/131	36/38	162	47,5
Mandipropamida	108/109	110/102	180	162
Ciromazina	60/61	81/80	95	125
Metomil	108/116	107/105	177	225
Pimetrozina	30/28	81/75	45	120
Acetamiprido	103/104	99/103	167	155
Buprofezina	98/97	96/96	167	166
Lucifenuron	68/67	64/63	-	-
Tiametoxan	104/98	70/69	165	112

Source: Prepared by the authors

The treatment with calcium hydroxide solution (pH=12.3) showed better recovery factors for most analytes, except for Acibenzolar-S-Methyl that did not recover well in any of the treatments. Possibly this is due to methylation of the structure of the sulfur compound, which makes it difficult to extract in acetonitrile. Thus, validation proceeded based on treatment 02.

2.13 FORTIFICATION TRIALS TO EVALUATE METHOD ACCURACY

For the study of the accuracy of this analytical method, fortification tests were performed in order to verify the recovery factor of the compounds under study. Thus, five fortifications of the "reference blank" samples were performed at two different concentration levels, for a total of 10 assays.

Each fortification level was injected twice, totaling an n = 10 (5 extractions x 2 injections).

For the modified QuEChERS method extraction procedure, 15.00 g of homogeneous soil was weighed into polypropylene tubes (Falcon type), with a screw cap (50 mL capacity). Each sample was then moistened with 5 mL of Milli-Q water and shaken vigorously for 30

seconds in Vortex. The fortification was added at the two levels, using calibrated pipettes of 0.5 mL and 1.0 mL, at the concentrations: of 0.200 µg. mL-1 for all pesticides contained in the fortification solution.

After fortification, the samples were homogenized by vortex mixing for 30 seconds and kept at 20°C for 15 minutes. Research by PINTO et al. (2010), indicates that it is critical that there is enough time for the sample with the analytes for the solvent to evaporate and, this way, there is more interaction between the compounds and the matrix. According to the author, this step brings the test closer to the reality of interaction that occurs with the samples in the field.

Then, using a volumetric pipette, pour 5 mL of saturated calcium hydroxide solution pH 12.3 into each tube, and after closing them, they were vortexed for 30 seconds. Allowing to react for 10 minutes at a standstill. Then, 15 mL of Lichrosolv grade acetonitrile (for residue analysis) was added to each alloy and stirred again for 30 seconds.

Were added 1.5 of sodium chloride (NaCl) and 6.0 g of MgSO4 (anhydrous magnesium sulfate) in each and stirred for another 30 seconds in a vortex to achieve the greatest possible interaction between the liquid extract and the solid reagents.

In a vial with a capacity of 2 mL, a dilution was made in the proportion 1:1 (v/v), in which 1.0 ml of the extract obtained after extraction and 1.0 mL of mobile phase were added, followed by LC-MS/MS analysis. Finally, dilutions of the final extracts were made in a 1:1 (v/v) ratio in the mobile phase (ultrapure water). The recovery of the compounds was evaluated at concentrations of 1 and 2 µg.kg-1 soil for all pesticides in the fortification solution.

The recovery results were interesting in both treatments. However, treatment 02 proved to be more effective in extracting more of the pesticides, with recoveries in the range of 64 to 110%, Except for Acibenzolar-S-Methyl, whose recoveries were more significant in treatment 1. The results of the experiments to evaluate the best extraction and cleaning method are shown in Table 1 - Trials performed for the optimization of the extraction step.

The clean-up step showed no significant improvement in the results. Thus, it was decided to proceed to the validation stage using treatment 02 without the clean-up step.

2.14 VALIDATION METHOD

The extraction method was validated according to the Analytical Quality Assurance Guide. The values established in this manual meet the requirements of Decision 2018/657 (*European Commission/SANTE*, 2018).

The following parameters were evaluated: selectivity; matrix effect; linearity; recovery; limit of detection (LOD); limit of quantification (LOQ) and repeatability. In the proposed

method, selectivity was evaluated by analyzing five replicates of the sample extracts from tomato soils. 0, 0.5, 1.0, 1.5, and 2.0 times the MRL established for each analyte. Cochran's test was used to evaluate the homogeneity of the variances obtained for each concentration level. The calibration data were evaluated by ordinary linear regression in case of homoscedasticity or weighted linear regression in case of heteroscedasticity.

For the extraction of the samples, the same reference soil from the initial tests was used. In the proposed method, selectivity was evaluated by analyzing five repetitions of the sample extracts from the tomato plantation soil. In 10 centrifuge tubes, Falcon type, 15 g were weighed and 1mL of the working solution was added. In tubes numbered 1 and 5, 1mL of the fortification solution with level 1 working solutions was added to each tube, and in tubes numbered 6 through 10, 1mL of the fortification solution with level 2 working solutions was added to each tube, plus a blank test tube with unfortified propoxur quality control. The 11 tubes received all the steps that were used in treatment 2, and then 1 mL of the extract was transferred to a vial and 1mL of MeOH (mobile phase component) was applied. Then, 5µL was injected into the Ultra Performance Liquid Chromatograph coupled to the sequential mass spectrometer, under the same conditions adopted for assays 3 and 4 of treatment 2. Em seguida, 5µL foi injetado no Cromatógrafo Líquido Ultra Performance acoplado ao espectrômetro de massa sequencial, sob as mesmas condições adotadas para os ensaios 3 e 4 do tratamento 2.

The effect of the matrix was evaluated by comparing the slope of the analytical curve in the matrix extract with the slope of the analytical curve in the solvent, using the F-test (Fisher Snedecor). Then, Student's t-test was applied to determine the statistical equivalence between the slopes of the analytical curves in the solvent and the matrix.

The LOD and LOQ were calculated by the signal-to-noise ratio of the equipment. LOD was the concentration equivalent to three times the noise and LOQ was the concentration equivalent to six times the noise. Recovery and repeatability of the method were performed with soil samples spiked at two levels: 0.5 to 1.0 equivalent to 5 times the MRL of each analyte, with five repetitions for each level. The mean recovery and relative standard deviation (RSD) were calculated for each level. Sample analysis. Field samples were kindly provided by producers in the state of Rio de Janeiro, Brazil, were analyzed by the validated method.

The calcium hydroxide modified QuEChERS method showed better recovery results than the original QuEChERS method for most analytes, especially for Abamectin, Acetamiprid, Azoxystrobin, Buprofezin, Diafenthiuron, Mandipropamid, Pymetrozine, Cyromazine, Methomyl, Pymetrozine, Lucifenuron, and Thiametoxan. With the modified method (QuEChERS method with Ca (OH)2), the recovery values obtained were within the acceptable

range of 70-120% (ANVISA, 2018). Treatment 2 had only one recovery result outside the acceptable range of 80-110%, Acibenzolar-S-Methyl (37%). The clean-up step with dispersive SPE did not promote significant improvements in recoveries. The SPE cleanup step turned out not to be necessary because the first extract obtained was clear and showed acceptable recoveries for the compounds of interest, as presented in Table 2.

Therefore, the extraction method chosen to follow in the validation process was the method based on treatment 2 (QuEChERS with calcium hydroxide) without the SPE clean-up step, using MgSO4, PSA, and C18.

The accuracy was calculated using the following equation and was expressed in percent recovery (INMETRO, 2007):

$$R\,(\%) = \frac{C_1 \,.\, C_2}{C_3} \times 100$$

Where

C_1 = Concentration determined in the fortified sample;

C_2 = Concentration determined in the unfortified sample;

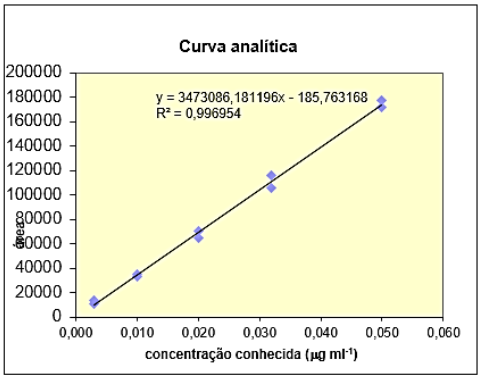
C_3 = Concentration used for fortification.

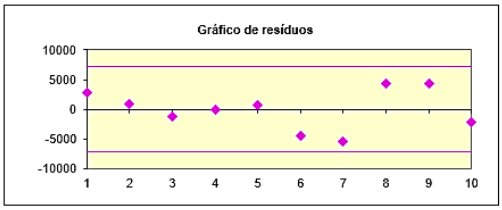
There was no interference with the same m / z and retention time of the analytes in the five repetitions performed with the matrix extract. Thus, it was possible to obtain the selectivity of the method. The spreadsheet for evaluation of the analytical curve - Validation of the multi-residue method by CLUE-MS/MS is shown in Figure 1.

Figure 3 - Analytical curve evaluation data - Multiresidue method validation by CLUE-MS/MS

Curva de Calibração			Cálculos				Avaliação dos resíduos – Homocedasticidade				
Concentração		Resposta	Média	Variância	Desv. Pad.	CV	Somatório	Maior	C de Cohran		Resultado
Pontos	µg ml^{-1}	área				%	Variância	Variância	C calculado	C teórico	homo/hetero
1	0,0030	13122,589	12122,1325	2001826,417	1414,8592	11,672	85267644,95	48703051,26	0,5712	0,841	Homocedástico
	0,0030	11121,676									
2	0,0100	33291,605	33882,109	697389,948	835,0988	2,465	ANOVA – da regressão e Teste de desvio da Linearidade				
	0,0100	34472,613					Fonte	G.L.	SQ	MQ	F
3	0,0200	70041,195	67492,4395	12992309,2	3604,4846	5,341	Regressão	1	3,35E+10	3,35E+10	2618,7840
	0,0200	64943,684					Resíduos	8	1,02E+08	1,28E+07	#
4	0,0320	105532,672	110467,3985	48703051,26	6978,7571	6,317	Total	9	3,36E+10	#	#
	0,0320	115402,125					Xmed=	0,0230			
5	0,0500	177742,578	174512,0155	20873068,13	4568,7053	2,618	Ymed=	79695,219			
	0,0500	171281,453					Se^2=	12786476,8	Resultado	Regressão	Significativa
Somatório	0,23000	796952,19					Se=	3575,82			
							n=	10			
							coef.linear=	-185,76317			
							coef.ang =	3473086,18120	n = número de observações		
							alfa=	0,05	u = número de níveis		
							u=	5	2 = número de parâmetros estimados		
							r =	0,998476			
							R^2 =	0,996954			
							p =	0,05			

valor-p	Sign.
2,355E-11	p < 0,05





Source: Cardoso, et al (2010 - MassLynx® Software

The performance characteristics of the optimized method, the working range, the values of the correlation coefficients (r), and determination coefficients (R2) for analytical curves obtained for each analyte are shown in Chart 3.

Chart 1 - Summary of the evaluation results - Correlation (r) and determination coefficients (R2)

Substance	VALIDATION OF THE ANALYTICAL CURVE	
	r	R^2
Azoxistrobina	0,9995	0,9989
Boscalida	0,9982	0,9964
Carbendazim	0,9996	0,9993
Clorantraniliprole	0,9996	0,9991
Clotianidina	0,9963	0,9925
Diafentiuron	0,9993	0,9986
Difenoconazol	0,9988	0,9975
Dimetomorfe	0,9991	0,9982
Espinetoram	0,9973	0,9947
Espinosade	0,9987	0,9974
Fenuron	0,9986	0,9973
Imidacloprido	0,9995	0,9990

Indoxacarbe	0,9981	0,9961
Metalaxil M	0,9998	0,9997
Metoxifenozida	0,9969	0,9939

Source: elaborated by the author

The matrix effect was not evaluated for soil validation, and was found to be significant for all pesticides studied. All analyzed substances showed homoscedastic behavior in the working range of 0.0032 to 0.0500 µg/mL.

It is observed that for most analytes, the coefficients of determination (r2) were close to one, showing good linearity, indicating a homoscedastic dispersion profile (constant variation of experimental errors for different observations) for most analytes, allowing the standard curves to be evaluated by linear regression using the ordinary least squares method. The weighted linear fits (1 / x) were made using MassLynx® software. The values obtained for LOD and LOQ, as well as the signal-to-noise ratio (chart 4), met the criteria established by the National Health Surveillance Agency (ANVISA, 2018) for these analytes, confirming that the optimized method is suitable to meet the legislation in force in Brazil. However, to comply with European legislation, the obtained LOD and LOQ need to be revised, as they are very close to the established maximum level (*European Comission*, 2018).

Chart 2 - Validated substances in the soil matrix, with respective limits of quantification and corresponding signal-to-noise ratio

Substance	VALIDATION OF THE ANALYTICAL CURVE	
	LOQ (mg/kg)	Ratio Signal-to-Noise Ratio
Azoxistrobina	0,0066	538,39
Boscalida	0,0076	30,11
Carbendazim	0,0055	166,53
Clorantraniliprole	0,0075	276,84
Clotianidina	0,0064	496.4
Diafentiuron	0,0038	72,37
Difenoconazol	0,0077	38,83
Dimetomorfe	0,0072	27,62
Espinetoram	0,0074	10729,08
Espinosade	0,0078	1757,72
Fenuron	0,0080	1630,64
Imidacloprido	0,0132	207,19
Indoxacarbe	0,0062	171,61

Metalaxil M	0,0072	1104,23
Metoxifenozida	0,0074	327,56

Source: Prepared by the authors

It was possible to establish the LOQ for the substances at the validated fortification level, as they had a signal-to-noise ratio greater than 10.

2.15 ACCURACY (RECOVERY RATE) AND PRECISION (REPEATABILITY).

For the recovery rate and repeatability study, the soil sample A1BR05 was fortified with different volumes of the fortification stock solution, in five replicates, after extraction, the volume of 1 mL was removed and diluted 1:1 with methanol (MeOH) for subsequent chromatographic analysis by UHPLC-MS/MS. This fortification concentration corresponds to the theoretical QL concentration. Each replicate was injected twice. The obtained results of the accuracy-recovery are described in Chart 5.

Injection concentrations:

- Level 1: 0.00323 µg/mL which corresponds to 0.0067 mg/kg,

- Level 2: 0.00625 µg/mL which corresponds to 0.0133 mg/kg,

Chart 3 - Resultados obtidos da exatidão – Recuperação

Substance	VALIDATION OF THE ANALYTICAL CURVE			
	Nível 1		Nível 2	
	Conc. (mg kg^{-1})	. (%)	Conc. mg kg	Rec. (%
Azoxistrobina	0,0066	95,4	0,0142	106,3
Boscalida	0,0076	111,5	0,0149	110,5
Carbendazim	0,0055	80,4	0,0125	94,2
Clorantraniliprole	0,0075	109	0,0153	114
Clotianidina	0,0062	92,1	0,0146	109,9
Diafentiuron	0,0038	53,7	0,0042	32,5
Difenoconazol	0,0077	112,2	0,0148	111
Dimetomorfe	0,0072	105,4	0,015	111,5
Espinetoram	0,0074	103,5	0,0149	111,5
Espinosade	0,0078	114,4	0,0159	118,9
Fenuron	0,008	115,9	0,016	119,4
Imidacloprido	0,0063	92,1	0,0143	106,4
Indoxacarbe	0,0062	90,3	0,0135	101
Metalaxil M	0,0072	105,1	0,0155	116,6

Metoxifenozida	0,006	88,1	0,0146	109,6

Source: elaborate by the author

The recovery results are within the acceptable range (70-120%). The method showed good repeatability for most of the compounds investigated, with RSD values of less than 20%.

All studied compounds met the criteria recommended by the European Commission (2018), except for the pesticide Diafentiuron, because it did not provide recovery in the acceptable range (70 to 120 %) in neither of the two levels, making it impossible to validate.

After validation, the method was used for the quantitative determination of pesticide content in soil samples taken from the regions where tomatoes are planted. The results obtained are plotted in Table 6 below:

Tabela 4 - Resumo dos resultados das amostras reais de solo colhidas nas áreas de plantio do tomate (mg/Kg de solo)

Agrotóxico	A1			A2			A3			A4			A5			A6			A7		
	0-5	5 10	10 20	0-5	5 10	10 20	0-5	5 10	10 20	0-5	5 10	10 20	0-5	5 10	10 20	0-5	5 10	10 20	0-5	5 10	10 20
1. Azoxistrobina	0,003									0,0025						0,009	0,0035	0,006	0,06	0,012	0,003
3. Boscalida																Traços					
4. Carbendazim																0,0065	0,0065	0,0045	0,0085	0,003	0,002
5. Clomazona *														X*							X*
6. Clorantraniliprole										0,036	0,03	0,01	X	X	X	0,071	0,035	0,039	0,223	0,073	0,073
7. Clotianidina										0,027	0,013	X				X	Traços	X	0,0185	0,021	0,021
8. Diafentiuron										0,0255	0,007					X					
9. Difenoconazol	0,003									0,038	0,0065	X							0,0285	0,006	X
10. Dimetomorfe				0,0105	Traços	X				0,48	0,117	0,019				0,096	0,015	0,084	0,0275	0,006	0,002
11. Espinetoram																X	X	X	X		
12. Espinosade A																0,002	X	X			
13. Espinosade D																X	X	X	X	X	X
14. Fenuron	X						Traços	Traços	X	X	0,002	Traços	Traços	Traços	X	X	X	X	X	X	X
15. Imidacloprido	X			Traços		X										0,008	X	0,004	0,006	0,003	X
16. Indoxacarbe										0,0235	0,002					0,0015					
17. Metalaxil M	Traços			Traços	Traços					0,0085	0,002					0,024	0,038	0,065	0,001	X	
18. Metoxifenozida										0,1415	0,0275	0,003				0,0105	0,0115	0,01			
19. Tiametoxan										0,0315	0,005	Traços				0,0225	0,002	0,008	0,0255	0,038	0,03

Note: A1 to A6 (Tomato Growing Areas) - Source: Authors' work.

Residues of the pesticides shown in Table 6 were found. The pesticide phenuron was found in all soil samples except those from areas A1 and A2. This pesticide is one of the excluded or unregistered in Brazil, as shown in table 7. However, the concentrations of this compound found in the samples were classified as trace, i.e. below the detection limit of the analytical method.

Regarding the pesticides azoxytrobin and carbendazim, the situation in areas 6 and 7 is worrying, mainly because these pesticides are not authorized by ANVISA for application in tomato planting, as shown in Chart 7

Chart 5 - Concentrations of pesticides NOT AUTHORIZED for tomato application found in

the analyzed soils

Agrotoxic	A6			A7		
	5-10	10-20	10-20	0-5	5-10	10-20
Azoxistrobina	0,0090	0,0035	0,006	0,060	0,012	0,003
Carbendazim	0,0065	0,0065	0,0045	0,0085	0,003	0,002

Source: elaborate by the author

The optimized multi-residue method proved to be selective and accurate over the range studied, allowing the simultaneous analysis of the substances: Azoxystrobin, Boscalid, Carbendazim, Chlorantraniliprole, Clothianidin, Difenoconazole, Dimethomorph, Spinetoram, Spinosad A, Spinosad D, Fenuron, Imidacloprid, Indoxacarb, Metalaxyl M, Methoxyfenozide, Thiametoxan, with their respective limits of quantification (LOQ), included in the official Brazilian tomato monitoring program, as presented in Chart 8

Chart 6 - LOQS for pesticides-focus: µg kg-1

Agotóxico	LOQ (µg kg^{-1})
Azoxistrobina	7,0
Boscalida	7,0
Carbendazim	5,0
Clorantraniliprole	7,0
Clotianidina	7,0
Diafentiuron	7,0
Difenoconazol	7,0
Dimetomorfe	7,0
Espinetoram	7,0
Espinosade	7,0
Fenuron	7,0
Imidacloprido	13,0
Indoxacarbe	7,0
Metalaxil M	7,0
Metoxifenozida	7,0

Source: elaborate by the author

FINALS CONSIDERATION

The QuEChERS method, with minor modifications, was suitable for multi-residue extraction of the analytes in soils from the plantation of the, with clear extracts free of interferences (UPLC-MS / MS) . was adequate for the detection and quantification of these analytes in the matrix, with recovery values between 70 and 120% standard deviation less than 20%,%, limits of quantification between 7 and 13 µg.L-1 and limits of quantification between 2 and 4 µg.L-1, appropriate to meet the legislation in force. The results of the field test showed that the method is suitable for quantitative analysis of pesticides evaluated in soils derived from tomato planting within the working range.

The validated method is under the values suggested in the literature for the analysis of pesticide residues by chromatographic methods (*European Comission*, 2018). obtained from the characteristic mass fragments of each analyte, and quantitative, through the MRM acquisition mode. The optimized chromatographic conditions for determination by UHPLC-MS/MS allowed the identification and quantification of the study compound in an analysis time of less than 15 min, which contributes with a great gain as an analytical tool and for society as a whole.

In general aspects, all samples presented concentrations of pesticides allowed by the ANVISA monographs. However, the results obtained for the conventional planting, despite being within the required conformities, are higher than the values obtained for the sustainable and organic system plantings. Nevertheless, it serves as a warning about the presence of pesticides on society's table.

The use of pesticides Azoxystrobin and Carbendazim (unauthorized pesticides) for application in tomatoes brings concrete concern about something that was normally already expected, the deliberate use of pesticides to increase production, regardless of what the law says.If on the one hand, it is worrisome to find unauthorized pesticides in the samples, on the other, this demonstrates that the method validated by this work is highly effective, due to its ability to quantify even pesticides that are not authorized for use.

In addition to achieving very satisfactory results for the focus pesticides, this work was able to determine residues for 240 pesticides, between those authorized and those not authorized by ANVISA in Brazil, including coefficient of determination values greater than 0.99; LOQ values of 13 µg kg-1 for Spinosad and 7.0 µg kg-1 for the other pesticides. The method showed good precision, with RSD values < 20%, and accuracy, with recoveries between 70 and 120% for the vast majority of the compounds analyzed.

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