Determination of Abamectin in Soybean Roots by Liquid Chromatography Coupled to Tandem Mass Spectrometry

Eliezer de Oliveira, Bianca Ferreira da Silva, Carla Beatriz Grespan Bottoli

Instituto de Química, Universidade Estadual de Campinas (Unicamp), PO Box 6154, Campinas, SP, 13083-970, Brazil

Abamectin has been used in seed treatment to control plant-parasitic nematodes. In this work, foliar spray was performed as an alternative application method and a LC-MS/MS method employing QuEChERS for sample preparation was developed for the analysis of abamectin in soybean roots. For this, abamectin was applied to the leaves and the translocation of this pesticide from leaves to roots was evaluated. The method was validated and presented adequate selectivity. Matrix-matched was used as an approach to calibration. Good linearity of the analytical curve was obtained over the studied range of concentrations from 0.10 to 1.0 mg kg\(^{-1}\), with a determination coefficient of 0.995. The limit of detection was 0.05 mg kg\(^{-1}\), and the limit of quantification was 0.10 mg kg\(^{-1}\). Recoveries were in the range of 99 to 106% and RSD < 20%. Finally, root samples after foliar spray were analyzed, and abamectin was not detected.

Keywords: abamectin, liquid chromatography, mass spectrometry, QuEChERS, soybean roots

INTRODUCTION

Plant-parasitic nematodes (PPN) cause extensive losses to crop yield worldwide. Some species of PPN attack roots to obtain their nutrients, affecting plant health and leaving plants vulnerable to diseases. To control root-parasitic nematodes on soybean crops in Brazil, abamectin is authorized by the "Ministério da Agricultura e Pecuária" (MAPA) for use as a nematicide by seed treatment. Abamectin is a macrocyclic lactone, which is produced by fermentation of the soil bacterium, \textit{Streptomyces avermitilis}. It belongs to avermectin family and consists of a mixture of components \(B_{1a}\) (at least 80%) and \(B_{1b}\) (not more than 20%) (Figure 1). Abamectin is a highly lipophilic substance and has low solubility in water. This nematicide has a broad-spectrum activity and is highly effective against nematode species, however, abamectin has poor mobility, and when applied by seed treatment, it tends to remain with the seed coat and bond to organic
contents in soil, leaving the plant seedling vulnerable after germination.\textsuperscript{2,3} Some approaches have been proposed to improve abamectin soil mobility, such as structural modifications,\textsuperscript{4} encapsulation of active compounds,\textsuperscript{5} and addition of adjuvants.\textsuperscript{6}

![Figure 1](image1.png)

**Figure 1.** The structure of abamectin is composed of ivermectin \( B_{1a} \) and \( B_{1b} \).

Another way to enable the use of abamectin to control root-parasitic nematodes would be to use an alternative application method, such as foliar spray. Foliar spray is an interesting method of application since it allows the control of other diseases and pests that affect different parts of the plant. For this, in the current study, abamectin translocation from leaves to roots on soybean plants after foliar spray application was evaluated. Since abamectin has limited plant systemic activity, an approach has been used to improve its uptake and translocation by adding a copper-based product.

Assessment of the performance of approaches, as described above, requires methods that indicate the movement of pesticides in different parts of a plant. For this, biological activity assays or analytical techniques have been used to evaluate the translocation/redistribution of pesticides in plants.\textsuperscript{7} Among these analytical techniques, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is a powerful tool in translocation evaluations that allows the quantification of compounds in different plant tissues and the comparison of their concentrations. The LC-MS/MS provides high sensitivity and selectivity for pesticide residue analysis and several methods have been reported employing this technique. For evaluations of abamectin translocation by LC-MS/MS, studies have focused on movement from stems to leaves and fruits, or from roots to leaves, flowers and fruits using coconut palm,\textsuperscript{8} walnut\textsuperscript{9} and apple trees.\textsuperscript{10} However, to our knowledge, previous studies have not evaluated abamectin translocation from leaves to roots in soybean plants by LC‒MS-MS.

Usually, methods for abamectin determination in plant tissues by LC-MS/MS employ some sample preparation steps in the analytical scope, such as the QuEChERS method or solid-phase extraction (SPE).\textsuperscript{5-11} SPE involves the use of a cartridge, usually with C18 sorbent, which can represent a high-cost technique and lengthy method development. On the other hand, the QuEChERS method is high-throughput and user-friendly, has reduced time, and involves reduced sample amounts and organic solvents.\textsuperscript{12} Because of these advantages, an LC-MS/MS method employing QuEChERS for sample preparation was developed for the analysis of abamectin in soybean roots after foliar spray application. This method will be used to study the translocation of abamectin with a copper-based product from leaves to roots.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Abamectin reference standard (purity > 90\%) was purchased from Sigma Aldrich (Buchs, Switzerland). Sodium hydrogen citrate sesquihydrate \((\text{C}_6\text{H}_6\text{Na}_2\text{O}_7\cdot1.5\text{H}_2\text{O})\) and ammonium formate \((\text{CH}_5\text{NO}_2)\) were provided by Sigma Aldrich (Steinheim, Germany). Anhydrous magnesium sulfate \((\text{MgSO}_4)\), anhydrous
sodium acetate (NaC₂H₃O₂) and ammonium acetate (CH₃COONH₄) were obtained from J.T. Baker (Xalostoc, Mexico). Sodium chloride (NaCl) and formic acid (85% purity) were supplied by Synth (Diadema, Brazil). Glacial acetic acid (CH₃COOH) was provided by Mallinckrodt Baker (Paris, USA). HPLC grade acetonitrile and methanol were purchased from Supelco (Darmstadt, Germany). Primary secondary amine (PSA) was obtained from Agilent Technologies (Santa Clara, USA) and tri-sodium citrate 2-hydrate (C₆H₅Na₃O₇.2H₂O) was provided by Vetec (Rio de Janeiro, Brazil). The commercial products used in the treatments included Vertimec® 18 EC (1.8% abamectin) supplied by Syngenta, and a copper-based product (3.5% Cu) supported by Satis (Araxá, Brazil). Ultrapure water was obtained from a Direct-Q ultrapure water purification system (Millipore, Molsheim, France).

A standard stock solution of 200 mg L⁻¹ and a working standard solution of 100 mg L⁻¹ were prepared in methanol. The working standard solution was used as the spiking solution in fortification studies. For the analytical curve, three standard solutions of 200 mg L⁻¹ and three working standard solutions of 100 mg L⁻¹ were prepared in methanol. The independent standard solutions of the analytical curve were prepared by spiking of working standard solutions in blank root samples. All the prepared standard solutions were stored at -18 °C.

**Sample treatment**

The roots were removed from the plants, washed immediately with water, and dried under a nitrogen stream for 12 hours. The roots were homogenized and ground using liquid nitrogen. The homogenized samples were stored at -18 °C until analysis.

**Sample preparation - QuEChERS method**

Four procedures of the QuEChERS method were evaluated in fortified samples of soybean roots. One gram of homogenized sample was weighed into 50 mL Teflon centrifuge tubes, and an aliquot (100 µL) of standard solution was added. Then, 9.9 mL of acetonitrile was added, and the tubes were shaken for 1 min. In the procedures involving QuEChERS, both acetate and ammonium acetate, were used in acetonitrile acidified with acetic acid in 1% (v/v). All procedures were treated as shown schematically in Figure 2.

![Figure 2](image)

**LC-MS/MS analysis**

Chromatographic separation conditions were adapted from a previous work developed by Perioto et al. for the analysis of abamectin in dende. Chromatographic separation was performed using a Waters
Alliance 2695 high-performance liquid chromatograph (Milford, USA). The analysis was accomplished on a Nova-Pak C18 column (150 mm x 3.9 mm, 4.0 μm, Waters) that was operated at 40 °C. The separation was performed in isocratic mode, and the mobile phase consisted of 15% solvent A (water, pH 4.0) and 85% solvent B (methanol), both modified with 10 mmol L⁻¹ ammonium formate. The injection volume was 10 μL, the flow rate was set at 1 mL min⁻¹, and the total runtime was 10 min.

Detection was performed using a Waters Micromass Quattro Micro API tandem mass spectrometer (Milford, USA) equipped with an electrospray ionization (ESI) source. The electrospray was operated in the positive mode, and the source parameters were capillary voltage 3.5 kV; cone voltage 20 V; source temperature 150 °C; and desolvation gas temperature 450 °C. The flow rates for desolvation gas and cone gas (N₂) were set at 1000 and 50 L h⁻¹, respectively. Detection was carried out in selected reaction monitoring (SRM) mode. The SRM data are shown in Table I. For instrument control and data acquisition, MassLynx software 4.1 was used.

<table>
<thead>
<tr>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Cone voltage (V)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>890.57</td>
<td>305.3</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>567.4</td>
<td></td>
<td>18</td>
</tr>
</tbody>
</table>

*a* Quantification and *b* Identification ion.

**Method validation**

The performance of the method was evaluated under the experimental conditions obtained in the final procedure. The parameters evaluated were selectivity, matrix effect, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and trueness.

For selectivity, a blank and a fortified extract at 1 mg L⁻¹ of soybean root were analyzed, and both chromatograms were compared.⁴¹ The matrix effect was evaluated by analyzing analytical curves of abamectin prepared in methanol and root extract at five concentration levels (25, 50, 100, 300 and 500 µg L⁻¹). The matrix effect was expressed quantitatively as demonstrated in Equation (1).⁵

\[
\text{Matrix effect} (\%) = \frac{\text{Analytical curve slope}_{\text{spiked matrix extract}} - \text{Analytical curve slope}_{\text{solvent}}}{\text{Analytical curve slope}_{\text{solvent}}} \times 100 \tag{1}
\]

The LOD and LOQ were defined by spiking blank samples at the lowest concentration that produced peaks with S/N ratios greater than 3 and 6, respectively. Furthermore, the LOQ was set as the concentration level with precision RSD less than 20%.⁴¹

Linearity was evaluated by preparing an analytical curve using matrix-matched calibration at five concentration levels (0.10, 0.25, 0.50, 0.75 and 1.0 mg kg⁻¹). Each concentration level was prepared in triplicate and injected in duplicate.⁴¹ For matrix-matched calibration, the spiking of blank root samples was performed before sample preparation. The data were treated by weighted least-squares regression (WLS).⁶

The trueness and precision of the method were evaluated by spiking blank samples of soybean roots at three concentrations (0.10, 0.50, and 1.0 mg kg⁻¹) in six replicates since no certified reference materials were available. The repeatability (intraday precision) and intermediate precision (interday precision) were expressed as the relative standard deviation (RSD) of the concentration for six successive analyses carried out on the same day (n = 6) and three different days (n = 18), respectively. The established criteria to accept intraday and interday precision were RSD lower than 20%.⁴¹
Analysis of roots obtained from plants treated with abamectin

For evaluation of abamectin translocation from leaves to roots when applied by foliar spray, a field trial was conducted at a greenhouse of Universidade Adventista de São Paulo (UNASP) located in Engenheiro Coelho city during February, March, and April of 2022. Soybean seeds were cultivated in plastic bags filled with a growth medium composed of a mixture of class B organic fertilizer and coconut fiber, at a ratio of 1 kg of fertilizer to 1 L of fiber. After germination, 4 seedlings were kept in each bag, constituting a single sample. When the plants reached phenological stages V3-V4, two treatments were applied by foliar spray: one containing abamectin (dosage of 18 g a.i. ha⁻¹) and another containing abamectin (dosage of 18 g a.i. ha⁻¹) + the copper-based product (3.5% Cu). Plants without any foliar spray were considered as controls. Root samples were collected at 4, 7, 14, 21, 28, 35, 42 and 49 days after treatments. All samples were collected in triplicate.

RESULTS AND DISCUSSION

Sample treatment

After the roots have been collected from the field, the roots were superficially rinsed with water to remove adhering soil and debris. As the plants were cultivated in soil, the roots were heavily soiled, which could have affected the sample preparation.

Optimization of the QuEChERS method

The QuEChERS method has been widely used in many pesticide analyses. In the original QuEChERS method proposed by Anastassiades et al.,¹⁷ 10 g of sample were used for the extraction procedure. Once low quantities of root samples are obtained from a plant, in the present work, the procedure was modified by reducing the sample quantity to 1 g. This strategy was applied with success in abamectin determination in dende and demonstrated by Periotto et al.¹³ For abamectin determination in soybean roots, different modifications of the QuEChERS method were investigated (Figure 3) and there were slight differences among the extraction procedures. Thus, the average areas obtained for each method were compared by ANOVA and were considered different ($p < 0.05$) at the 95% confidence level. The original method presented the highest analyte signal and was chosen for abamectin extraction. In addition, the original method presented the lowest reagent quantities and achieved the lowest RSD (1.43%).

Figure 3. Comparison of different QuEChERS method procedures in fortified soybean root samples.
**Method validation**

The absence of interfering peaks from endogenous compounds was checked by analyzing a blank and a spiked soybean root extract. The selectivity of the method was confirmed by the absence of interfering peaks at the retention time of abamectin in the blank soybean root extract (Figure 4). By using tandem mass spectrometry in selective reaction monitoring (SRM) mode, high selectivity was observed.

Despite the good selectivity of LC-MS/MS observed in Figure 4, endogenous compounds present in the sample extract may coelute, affecting the analyte ionization process. Either suppression or enhancement of analyte signals may occur, affecting the accuracy of quantitative data. Therefore, the matrix effect was evaluated according to Equation (1). The difference in the analyte signal in solvent and root extract can be seen in Figure 5 by comparing the slopes of the analytical curves. When the values of the matrix effect are less than -20% or higher than +20%, the matrix effect is considered significant. The matrix effect calculated was +20.8%, showing that abamectin presents a signal enhancement effect in soybean root extract. It was concluded that the components of the sample affect the analyte ionization in the source, and matrix-matched calibration was used as an approach to compensate for the matrix effect.
The lowest concentration that presented a calculated S/N ratio greater than 3 was 0.05 mg kg\(^{-1}\), and this point was defined as the LOD. The LOQ was 0.10 mg kg\(^{-1}\) with an S/N ratio greater than 6 and a RSD of 12.4%.

The analytical curve was obtained by plotting the peak area over the range of concentrations from 0.10 to 1.0 mg kg\(^{-1}\) at five calibration levels, and the lowest spike level of the analytical curve was the LOQ. Initially, the data were treated by ordinary least squares regression (OLS). However, as shown in Figure 6a, the residuals are heteroscedastic, and in this case, the standard deviation of the y-values increases with the concentration of the analyte. For this reason, the data were treated by WLS regression assuming 1/s as the weight. The visual inspection of the residual plot in Figure 6b allows us to verify the homogeneity of variances after treatment by WLS regression. Finally, the weighted regression parameters were estimated as follows: determination coefficient (r\(_w^2\)) 0.995, intercept (a\(_w\)) 0.723 and slope (b\(_w\)) 259.4.

![Figure 6](image)

The results of trueness and precision are summarized in Table II. The trueness and precision of the method were evaluated by spiking blank samples of soybean roots with abamectin at three concentrations. The method presented adequate trueness with recoveries ranging from 99 to 106%. The repeatability and intermediate precision were calculated using the RSD. Intraday RSD values in the range of 3 - 9% were obtained. Interday RSD values were all within 6 - 7%. These RSD demonstrate that among the criteria established, the intraday and interday precision of this method are acceptable (< 20%).

<table>
<thead>
<tr>
<th>Concentration (mg kg(^{-1}))</th>
<th>Recovery (%)</th>
<th>Intraday RSD (%)</th>
<th>Interday RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>106</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>0.50</td>
<td>99</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>1.0</td>
<td>105</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

**Method application**

The translocation of abamectin from the leaves to roots in soybean plants was evaluated by comparing the foliar spray application of commercial abamectin and a mixture of abamectin with a copper-based product. For this, 96 samples of soybean roots collected after the treatments by foliar spray application in a greenhouse were analyzed by the LC-MS/MS method described above. The presence of abamectin was not detected in any sample. Since the concentration level of abamectin in soybean roots was not found in the literature, it is not possible to conclude that these results are due to the low concentration of abamectin in the roots (lower than the LOD of method) or the limited plant systemic activity of abamectin. Research is being carried out to further explore the potential of this field.
CONCLUSIONS

An LC-MS/MS method for abamectin determination in soybean roots was established using the QuEChERS method for sample preparation. The method was validated, and the performance characteristics were acceptable. Matrix-matched calibration enabled compensation of the matrix effect and ensured accurate quantifications. The trueness and precision of the method were adequate, with recoveries ranging from 99 to 106%, intraday RSD values from 3 to 9% and interday RSD values from 6 to 7%. The method was applied to the determination of abamectin in soybean roots and all the samples were free of abamectin, at least above the detection limit. This method provides a strategy to study the translocation of abamectin in soybean plants.

Conflicts of interest

The authors declare that they have no financial conflicts of interest.

Acknowledgements

The authors acknowledge the Satis company and the "Universidade Adventista de São Paulo" (UNASP) for the use of the greenhouse located in Engenheiro Coelho, SP, Brazil.

The authors acknowledge financial support from the “Instituto Nacional de Ciência e Tecnologia em Bioanalítica” (INCTBio) grant numbers: FAPESP/INCT 2014/50867-3, CNPq 465389/2014-7. C. B. G. Bottoli acknowledges CNPq for a research fellowship [grant 309363/2018-7]. E. de Oliveira and B. F. da Silva acknowledge “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” - Brasil (Capes) - Finance Code 001 for a graduate fellowship [grants 88887.517929/2020-00 and 88887.339505/2019-00].

Graphical Abstract created with BioRender.com

REFERENCES


