LC-MS/MS method for estimating the exposure to neonicotinoid residues in pollinator attractive habitat adjacent to corn and soybean fields

Abstract: Recent research has suggested that neonicotinoid insecticides applied to crops can be detected in adjacent pollinator-attractive habitats and pollen collected by honey bees. Honey bees, native bees, and monarchs could be exposed to neonicotinoids through ingestion of contaminated pollen, nectar, and milkweed leaves. Although these studies indicate that neonicotinoids can be detected in pollinator-attractive habitats, the magnitude and extent of potential adverse effects to honey bees, native bees and monarch larvae is an active area of research. In this study, we developed and validated an innovative method to simultaneously evaluate concentrations of clothianidin, imidacloprid, and thiamethoxam, and two imidacloprid metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in plant foliage. The results indicate acceptable ranges for the recovery, low and high quality control (between 75% and 110%), while the matrix effect indicates minimal ion suppression or ion enhancement for all analytes. The calibration curves were linear over the concentration ranges with $r^2$ at $> 0.998$. The data obtained established a validated, single extraction and LC-MS/MS analytical method for quantifying neonicotinoid concentrations to a method detection limit of 0.04 to 0.3 ng/g plant material. This method is comparable or in some cases better than existing methods that require separate extraction and/or LC-MS/MS methods. We are using the method to analyze leaf and pollen samples collected in 2017 from habitat patches adjacent to corn and soybean planted with neonicotinoid-treated seeds. This data will provide insights on spatio-temporal variability of neonicotinoid concentrations in Midwestern agroecosystems and help inform pollinator risk assessments.

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Recent research has reported that neonicotinoid insecticides applied to crops can be detected in adjacent pollinator-attractive habitats and pollen collected by honey bees. Honey bees, native bees, and monarch butterfly larvae could be exposed to neonicotinoids through ingestion of contaminated pollen, nectar, and milkweed leaves. Although these studies indicate that neonicotinoids can be detected in pollinator-attractive habitats, the magnitude and extent of potential exposure and adverse effects to honey bees, native bees, and monarch larvae is an active area of research. In this study, we developed and validated an innovative method to simultaneously evaluate concentrations of clothianidin, imidacloprid, and thiamethoxam, and two imidacloprid metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in plant foliage. The method provides a validated, single extraction and LC-MS/MS analytical approach for quantifying neonicotinoid concentrations to a method detection limit of 0.04 to 0.3 ng/g plant material. The method provides acceptable ranges for recovery (between 75% and 110%) and minimal ion suppression or enhancement for all analytes. The calibration curves were linear over the concentration ranges with r² at > 0.998. This method’s performance is comparable, or in some cases superior, to existing methods that require separate extraction and/or LC-MS/MS methods. We are using the method to analyze leaf and pollen samples collected in 2017 from habitat patches adjacent to corn and soybean fields planted with neonicotinoid-treated seeds. This data will provide insights on spatio-temporal variability of neonicotinoid concentrations in Midwestern agroecosystems and help inform pollinator risk assessments.

**Abstract**

The Natural Resources Conservation Service (NRCS) and public citizens are questioning whether adding pollinator and monarch habitat in close proximity to farm fields is harmful to visiting pollinators. We are measuring the neonicotinoid exposure in-field pollinator habitat in planted with neonicotinoid-treated corn and soybean seeds, to determine any potential risk to visiting bees or to monarch larvae. In order to complete this, a method was developed and validated for evaluating the compounds of interest in the foliage matrix and pollen.

**Example Table**

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Mode of action</th>
<th>Water Solubility (ppm)</th>
<th>Log Kow</th>
<th>Henry’s Constant</th>
<th>Degradation rate (half-life in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hydrolysate (pH 7)</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>nACHR agonist</td>
<td>610</td>
<td>0.57</td>
<td>4.1x10⁻¹⁰⁴</td>
<td>33 to 44</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>nACHR agonist</td>
<td>327</td>
<td>0.7</td>
<td>8.4x10⁻⁷</td>
<td>Stable</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>nACHR agonist</td>
<td>4100</td>
<td>-0.13</td>
<td>1.0x10⁻¹⁰⁴</td>
<td>572 to 643</td>
</tr>
</tbody>
</table>

**Chemical Information**

**Analytes of Interest**

- Imidacloprid
- Clothianidin
- Thiamethoxam
- 5-hydroxy imidacloprid
- Imidacloprid olefin
- Clothianidin-4s
- Clothianidin-4d

**LC-MS/MS Method**

**Method Validation Results**

- Extraction method was able to recover >78% of analytes with minimal to no matrix effect.
- LC-MS/MS method was able to quantify all analytes of interest in a single run with MDL equal to, or below those found in literature.
- Method will allow analysis of field samples at a relatively fast throughput.

**On-Going Work**

- Quantifying foliage samples collected in 2017 and 2018 habitat patches integrated into row crop fields.
- Quantifying pollen samples from 2017 and 2018 field seasons taken from bees found in corn and soybean fields with and without in-field habitat patches.
- Using data to determine spatio-temporal variability of neonicotinoid concentrations in Midwestern agroecosystems.

**Acknowledgements**

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