**Biomarker Profiles of Echinacea Species Using Pressure Cycling Technology and MALDI-TOF Mass Spectrometry**

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**Abstract**

Echinacea, a genus of flowering plants in the daisy family, are well known for their medicinal properties and have been the subject of increasing research interest. The ability to rapidly, reproducibly, and accurately quantify phytochemicals is essential for the development of standardized extracts and for the quality control of herbal products. In this research, pressure cycling technology (PCT) was combined with MALDI-TOF mass spectrometry (MS) to develop a rapid method for the extraction and analysis of phytochemicals from Echinacea species. The method was validated for the extraction and analysis of E. purpurea leaf, seed, and root extracts, and the technique was successfully extended to E. angustifolia leaf, seed, and root for the first time. The results indicate the potential to extend the method to additional species and, with further method refinement, a potential to automate or miniaturize the approach for high-throughput screening.

**Study Materials and Instrumentation**

**Echinacea purpurea** and **Echinacea angustifolia** were purchased from Nature’s Resource (Dry extract of E. purpurea, lot #010904 AHP; Scotts Valley, CA, www.herbal-aid.com). All samples were bacterially authenticated by AFST.

**Chemical Information**

**Table 1.** Sample preparation and extraction conditions.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Extraction Method</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Pressure (psi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Pressure Cycling</td>
<td>2</td>
<td>120</td>
<td>20,000</td>
</tr>
<tr>
<td>Seed</td>
<td>Pressure Cycling</td>
<td>2</td>
<td>120</td>
<td>20,000</td>
</tr>
<tr>
<td>Root</td>
<td>Pressure Cycling</td>
<td>2</td>
<td>120</td>
<td>20,000</td>
</tr>
</tbody>
</table>

**Results**

Figures 2 and 3 represent the Echinacea purpurea and E. angustifolia spectra for seeds, leaves, and roots. Extracts were analyzed using Echinacea as the MALDI matrix, and the samples were analyzed in reflection mode using a 35 ps laser and 100 Hz scan rate. The spectra for the Echinacea purpurea leaf were unique to the species (Figures 5 and 6), indicating that the product was probably not composed entirely of leaf tissue. The Nature’s Resource dietary supplement spectrum was also very similar to the E. purpurea seed spectrum (Figures 5 and 6). The E. angustifolia leaf, seed, and root extracts were analyzed using reflectron mode (mass resolution = 5,000) as an applied baseline magnesium 40 U MALDI mass spectrometry. Table 2 summarizes key TOF MS parameters.

**Conclusions**

This study demonstrated the utility of combining two rapid techniques for obtaining characteristic profiles of small molecule, pressure-cycling technology and MALDI-TOF mass spectrometry. The results of this study indicate the potential of molecular weight compounds is effectively extracted from plant tissue using PCT. Although we are currently evaluating PCT and its potential for plant products, PCT shows promise to be a rapid and reproducible means of extracting phytochemicals from complex plant and finished products. In PCT/MALDI-TOF analysis, samples can then be directly analyzed using MALDI-TOF with very little further processing necessary. The combined techniques held promise in the species differentiation of Echinacea, product quality, and control of all quality, control, and regulatory implications.

**Acknowledgments**

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