Development and Validation of an Analytical Method for Quantitation of Alpha-pinene in Rodent Mammary Tissue by Headspace GC-MS

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Abstract

Alpha-pinene (AP), produced by pine trees and other plants, is the main component of turpentine and is used as a fragrance and flavor ingredient. Exposure to AP occurs via use of personal care and household cleaning products and in the lumber industry. Despite widespread exposure, toxicity data for AP are limited. The objective of this work was to develop and validate a method to quantitate AP in rat and mouse mammary tissue, a potential target tissue. In support of the National Toxicology Program toxicokinetics and toxicology studies.

Materials & Methods

Materials

Alpha-pinene (AP; CAS No. 80-56-8); John D. Walsh Company, Inc., Ringwood, NJ

GC-MS System and Software

Agilent 6890 GC coupled to an Agilent 5973N MSD (MSD) detector; Agilent Chemstation (Chemap, Santa Clara, CA)

Samples

Sprague Dawley (SD) and Harlan Sprague Dawley (HSD) rat mammary tissue; B6C3F1 mouse mammary tissue; Rat and mouse liver tissues generated from toxicokinetic and toxicology studies.

Sample Preparation

Alpha-pinene (AP) can be quantitated in rat and mouse mammary tissue using this simple headspace GC-MS method.

The method was successfully validated in female Sprague Dawley rat mammary tissue over the concentration range 100-50000 ng/g. Matrix standard curves were linear (r ≥ 0.99), and the percent relative error (%RE) values were ≤ 12% for standards at all levels. Small background peaks were detected in the matrix and method blanks, but the response was low and did not interfere with method performance. Absolute recovery was low (2%) likely due to high lipophilicity of AP. However, the limit of detection, determined from the standard deviation at the lower quantitation limit (100 ng/g), was 17.7 ng/g, demonstrating adequate sensitivity. Recoveries incorporating the IS were ≥90% at all concentrations.

Results – Method Validation

The method was successfully validated in female SD rat mammary tissue (Primary Matrix) over the concentration range 100-50000 ng/g. Matrix standard curves were linear (r ≥ 0.99) and %RE ≤ 12% for standards at all levels. Small background peaks were detected in the matrix and method blanks, but the response was low and did not interfere with method performance.

Validation Design

Linearity: 6-point calibration curve in female SD rat mammary tissue over the range 100 - 50000 ng/g. Matrix standard curves were linear (r ≥ 0.99). Intra- and inter-day precision (% relative standard deviation, RSD) and accuracy (mean % of true concentration) were calculated for 6 replicates at the lowest concentration level to define LLOQ and LOD. Recovery: Single ion monitoring (SIM); Acquisition Mode: Positive SIM; Software: Agilent ChemStation (Chemap, Santa Clara, CA)

Results (cont’d)

<table>
<thead>
<tr>
<th>Concentration (ng/g)</th>
<th>Mean Found Conc. (ng/g)</th>
<th>% RSD</th>
<th>Mean Conc. Found (ng/g)</th>
<th>% RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>249.6 ± 1.9</td>
<td>1.6%</td>
<td>250.0 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>2490</td>
<td>2381 ± 2.0</td>
<td>1.9%</td>
<td>2490.0 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>255</td>
<td>2375.5 ± 2.6</td>
<td>1.1%</td>
<td>255.0 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td>238</td>
<td>2372.4 ± 2.1</td>
<td>0.8%</td>
<td>238.0 ± 0.7</td>
<td>0</td>
</tr>
</tbody>
</table>