An LC-MS/MS Method was Developed and Validated for the Quantification of an Intact 9kDa Peptide in Animal Plasma Samples from a Toxicology Study  
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Background
A small peptide has been developed for the restoration of the activity of the protein phosphatase (PP2A) tumor suppressor. PP2A is thought to play an important role in the dephosphorylation of proteins that control proliferation and the programmed cell death. Cancer cells have developed mechanisms to inhibit PP2A so that uncontrolled growth can occur. One of these mechanisms is by making a protein that masks PP2A and prevents it from regulating the cell cycle. The small peptide was monitored in multiple reaction monitoring (MRM) mode. To determine if the peptide can dephosphorylate the BPT protein and restore the function of PP2A.

Purpose
• Develop an LC-MS/MS method for the quantification of a large-intact peptide
• Overcome column secondary interferences by chromatography since the peptide had multiple basic residues
• Determine from 16 charge states which state had the best fragmentation response, and validate that charge state in solution

Methods
Samples were prepared with an aliquot of the compound and internal standard [35] mixed with 200 μL of HODOIN (pH 7.2) in each well. The plates were frozen for 24 h. The plates were then thawed, washed and dried. The plates were permeabilized and unbelted into a 96-well plate, then mixed with a reconstitution solution of 0.07% TFA. The reconstitution solution with HODOIN and TFA were tested and it was determined that a reconstitution solution of 0.07% TFA gives the best stability for the LLOQ quantification.

Samples were analysed using reverse phase chromatography on Phenomenex, micro Dia 100 μm I.D., 5 μm, Waters Acquity UPLC system equipped with a ZIC-HILIC 300 mm reverse phase and a Diode cell source.

Table 1. Matrix Effect.  
<table>
<thead>
<tr>
<th>Batch</th>
<th>LLOQ QC</th>
<th>High QC</th>
<th>LLOQ QC</th>
<th>High QC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mobile Phase A</td>
<td>Mobile Phase B</td>
<td>Mobile Phase A</td>
<td>Mobile Phase B</td>
</tr>
<tr>
<td></td>
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</table>

Conclusions
The LC-MS/MS method for the determination of the peptide in acidified plasma (LLOQ) and the high QC sample was developed and validated as specified in the validation protocol. Stability was demonstrated for the peptide in acidified plasma (LLOQ) samples and solutions under varying conditions of storage. The method was validated using a calibration curve range of 0.02 to 50 μg/mL and a LOQ of 0.02 μg/mL. The long-term stability evaluation indicated that the peptide is stable under these storage conditions for 118 days (LLOQ QC) and 126 days.

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