Determination of Nicotinic Acid in Human Plasma by a LC/MS/MS Method

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Introduction
Nicotinic acid participates in many physiological redox reactions, and is known to be an effective agent in treating hyperlipidemia. In biological samples nicotinic acid is commonly detected utilizing HPLC or capillary electrophoresis for separation followed by ultraviolet or, with derivatization techniques, fluorometric detection. The purpose of this study was to develop a method that provides a simple, rapid and specific route for the determination of nicotinic acid in human plasma. The many parameters and validation results of this method are presented.

Analytical Method Summary

- **Internal Standard**: d4-Nicotinic Acid
- **Analyte**: Nicotinic Acid
- **Column**: Synergi Polar RP, 150 X 2.0 mm, 4 µm, analytical column (Phenomenex, Torrance, CA)
- **Calibration Equation**: Power
- **Sample Extraction Method**: Protein precipitation by acetonitrile
- **Curve Range**: 20.0 to 10,000.0 ng/mL
- **Retention Times**:
  - d4-Nicotinic Acid: ~2.6 min
  - Nicotinic Acid: ~2.6 min
- **Solvent System**:
  - **Solvent A**: 0.1% formic acid in water
  - **Solvent B**: 0.1% formic acid in acetonitrile
- **HPLC Preservation**: Blend 91.5% Solvent A and 8.5% Solvent B during solvent delivery.
- **Quality Control Samples**: Included with one of the validation runs to determine precision and accuracy at the lower limit of quantitation.

Accuracy and Precision

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Mean (ng/mL)</th>
<th>SD 0.84</th>
<th>Mean Experimental Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Over-the-Curve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>8676.5</td>
<td>320.65</td>
<td>108.0</td>
</tr>
<tr>
<td>I.S.</td>
<td>504.8</td>
<td>8.35</td>
<td>99.1</td>
</tr>
<tr>
<td>Low Over-the-Curve</td>
<td>8302.0</td>
<td>344.63</td>
<td>102.4</td>
</tr>
</tbody>
</table>

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Reagent Blank High Std
Low Std
Plasma Blank High QCLow QC

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Stability


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Conclusions

- A robust, specific and simple assay for the analysis of nicotinic acid in human plasma has been validated.
- The method has been used to quantify human plasma for pharmacokinetic studies.

References