The major water soluble vitamins such as Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Nicotinic Acid and Nicotinamide), Vitamin B5 (Pantothenic Acid), Vitamin B6 (Pyridoxamine, Pyridoxal, and Pyridoxine), Vitamin B7 (Biotin), Vitamin B9 (Folic Acid) and Vitamin B12 (Cyanocobalamin) are essential nutrients required for normal body functioning that can either cannot be acquired from the diet and can be toxic in large doses and can cause significant medical issues when deficient.

A simple, sensitive, specific and accurate quantitative analytical method was developed for the chromatographic baseline separation and measurement of the water soluble vitamins in human serum. A Poroshell 120 EC-CN column on an Agilent 1260 HPLC and 6460 Mass Spectrometer system was used for this method.

### Introduction

The major water soluble vitamins such as Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Nicotinic Acid and Nicotinamide), Vitamin B5 (Pantothenic Acid), Vitamin B6 (Pyridoxamine, Pyridoxal, and Pyridoxine), Vitamin B7 (Biotin), Vitamin B9 (Folic Acid) and Vitamin B12 (Cyanocobalamin) are essential nutrients required for normal body functioning that can either cannot be acquired from the diet and can be toxic in large doses and can cause significant medical issues when deficient.

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### Results and Discussion

#### Precision

The inter-assay precision for the Water soluble vitamins were determined by extracting and quantifying five replicates of in-house tri-level QC material as shown. Folic Acid proved difficult to obtain consistent results due to break down in solution.

#### Sample Analysis

Three healthy adult male samples were analyzed for the presence of Water soluble vitamins and it was discovered that Biotin, Folic Acid and Cyanocobalamin were not present in the serum samples analyzed.

#### Table 1: MRM Acquisition Table- Quantifier Ion

<table>
<thead>
<tr>
<th>Compound</th>
<th>Curve Range (ng/ml)</th>
<th>LOD/LOQ (ng/ml)</th>
<th>S/N</th>
<th>%CV C1 (%)</th>
<th>%CV C2 (%)</th>
<th>%CV C3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>812</td>
<td>6.42</td>
<td>3.59</td>
<td>2.92</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>270</td>
<td>7.23</td>
<td>3.65</td>
<td>2.45</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>0.25 - 500</td>
<td>0.25</td>
<td>220</td>
<td>11.2</td>
<td>6.58</td>
<td>4.56</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>775</td>
<td>7.62</td>
<td>4.57</td>
<td>1.66</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>76.9</td>
<td>8.99</td>
<td>4.63</td>
<td>N/A</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>67.2</td>
<td>7.32</td>
<td>3.6</td>
<td>2.89</td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>0.25 - 1000</td>
<td>0.25</td>
<td>220</td>
<td>11.2</td>
<td>6.58</td>
<td>4.56</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>82</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>5 - 1000</td>
<td>5</td>
<td>119</td>
<td>8.36</td>
<td>4.22</td>
<td>4.15</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Cyanocobalamin</td>
<td>0.1 - 1000</td>
<td>0.1</td>
<td>577.7</td>
<td>11.3</td>
<td>8.2</td>
<td>4.65</td>
</tr>
</tbody>
</table>

### Conclusions

- Baseline separation of the water soluble vitamins was achieved within a 5 minute run on a Poroshell 120 EC-CN column. Other columns were evaluated but did not offer the same degree of fast separation.
- Excellent linearity (>998) of calibration curves with great accuracy, precision and reproducibility was also achieved down to low clinical levels for the majority of the analytes except for Folic Acid, Riboflavin and Nicotinic Acid.
- Further investigation into the best sample preparation will be carried out in order to achieve lower LOD and to achieve consistent results for all the clinically relevant water soluble vitamins.

### References


### Experimental

#### Reagents, Standards, Calibrators and Controls

The following standards were obtained from Isocisces:

- Biotin
- Folic Acid
- Riboflavin
- Biotin-13C4
- Pantothenic Acid
- Thiamine
- Pyridoxamine
- Pyridoxine
- Pyridoxamine-2H3
- Cyanocobalamin

The following standards were obtained from Cerillan:

- Niacinamide
- Niacinamide
- Thiamine-13C4
- Pantothenic Acid-13C15,H2

The following standards were obtained from Sigma-Aldrich:

- Folic Acid
- Methanol
- Formic Acid
- Ammonium Formate

#### Sample Preparation

- 200 µl of serum sample, calibrators, controls was taken and 10 µl ISTD at 1000 ng/ml were added to each.
- 400 µl of HPLC grade Water was added to each tube and vortexed briefly prior to centrifugation for 10 minutes at 13,000 rpm.
- The supernatant was transferred to MS vials for analysis.
- All in-house calibrators were prepared in DC Mass Spec Good Serum (Golden West Biological, Inc.)