

# LC/MS/MS Analysis of Water-soluble B Vitamins in Fortified Foods and Beverages

Jim Krol<sup>1</sup>, Loic Beyet<sup>2</sup>, Sneh Bhandari<sup>3</sup>, Jim Carlson<sup>4</sup>, Andre Schreiber<sup>5\*</sup>

<sup>1 2 3 4 5</sup> Applied Biosystems

\* Corresponding author - E-mail: [andre.schreiber@lifetech.com](mailto:andre.schreiber@lifetech.com); Phone: +1-905-660-9006;

Several publications describe the analysis of water-soluble B (WSB) vitamins, as standards or in clean matrices such as beverages, analyzed in a single LC/MS/MS injection. There is much interest in quantifying the WSB vitamins in more complex matrices, like food products. However, a major difficulty with a single WSB vitamin assay is that sample preparation depends both on the vitamin and the sample matrix. There is considerable interest in a generalized sample prep method for all WSB vitamins, but each vitamin's chemistry differs and a single extraction may not be appropriate. Another consideration is that the B Vitamins also have differing optimal electrospray ionization voltages. We present a method for identifying and quantifying the WSB vitamins in fortified foods and beverages. The method for all WSB vitamins and methods for individual vitamins utilize a C18 column, water/methanol gradient and positive ion ESI with differences in spray voltage and gradient. The experiment for analysis of all WSB vitamins is under 10 minutes. Two MRM transitions per analyte were used for analyte quantification and confirmation. The MRM transitions can also trigger a full-scan analyte product ion spectrum for library matching validation. Samples of vitamin-enriched water, a fortified energy beverage, a low-fat white milk, chocolate diet "shake", and multigrain cereal flakes were extracted with 0.1 N HCl (pH 1), 0.1% formic acid (pH 3), 0.1% formic acid / 5 mM ammonium formate (pH 5), or 5 mM ammonium bicarbonate (pH 9) as appropriate. Comparison of MS/MS results with microbiological methods shows that each extraction solution has its own efficiency, and extraction solution recovery of a spiked sample matrix varies with each vitamin, matrix, and pH. Major interferences are carbohydrates, various polymeric additives, and mineral content. In some cases, the MS/MS results are significantly higher than the immunoassay result and in some cases lower. However, the confirmatory ion ratios suggest the MS/MS results are valid. Lower results from the mass spectrometric analysis may be due to ionization suppression or increased specificity for the analyte over the microbiological assay. The low cost, routine MS/MS system gives linear standard curves for the WSB vitamins with from 0.5 to 1000 ng/mL using a 10  $\mu$ L injection. LODs of less than 1 ng/mL were obtained for each analyte. The fortified liquid samples were diluted between 10- and 50-fold and the cereal extract was diluted 1,000-fold to bring the concentration range of the analytes within the range of the standard curve. Additional sensitivity might be gained by increasing the sample dilution factors as this will also dilute away the suppression effects of the matrix. Dilution should simplify the sample preparation and make the analysis more reproducible. The current method is designed for the analysis of fortified food products, but the potential sensitivity gain suggests that the analysis of WSB vitamins at natural levels may be possible. Faster scanning, more sensitive instruments may provide a means to do faster chromatography and therefore improve sample throughput. The potential of additional sensitivity suggests that analysis of WSB vitamins at natural levels may be possible.

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