Sensitive quantitation of water-soluble vitamins in human serum

In this technical note, the combination of a rapid sample preparation procedure consisting of a protein precipitation with a robust and sensitive LC-MS/MS method using the SCIEX QTRAP 6500+ enabled accurate quantitation of 8 water-soluble vitamins extracted from human serum. Excellent linearity was observed across clinically relevant concentrations. Low-ng/L level sensitivity was achieved at the lowest calibrator with signal-to-noise ratios (S/N) of 207:1 for thiamine, 87:1 for riboflavin, 69:1 for nicotinamide, 28:1 for nicotinic acid, 118:1 for pantothenic acid, 9:1 for biotin, 11:1 for cyanocobalamin, and 16:1 for folic acid, demonstrating the quantitative performance of the assay.

## Key benefits of water-soluble vitamin analysis from human serum using the QTRAP 6500+ system

Low-ng/mL level sensitivity and excellent quantitative performance: Sensitive quantitation of water-soluble vitamins was performed with excellent precision (0.4% for thiamine at 0.25 ng/mL, 1.6% for riboflavin at 0.41 ng/mL, 2.7% for nicotinamide at 0.07 ng/mL, 11.3% for nicotinic acid at 0.1 ng/mL, 0.4% for pantothenic acid at 0.5 ng/mL, 6.2% for biotin at 0.05 ng/mL, 5.0% for cyanocobalamin at 10 ng/mL and 1.7% for folic acid at 1.7%

Rapid sample preparation: Water-soluble vitamin complexes were extracted from human serum samples using a protein precipitation procedure

Chromatographic separation: optimized LC conditions enabled fast (5 minutes) chromatographic separation of 8 water-soluble vitamins

Excellent linearity: calibration curves for the 8 water-soluble vitamins showed r^2 values above 0.99 across the calibration range
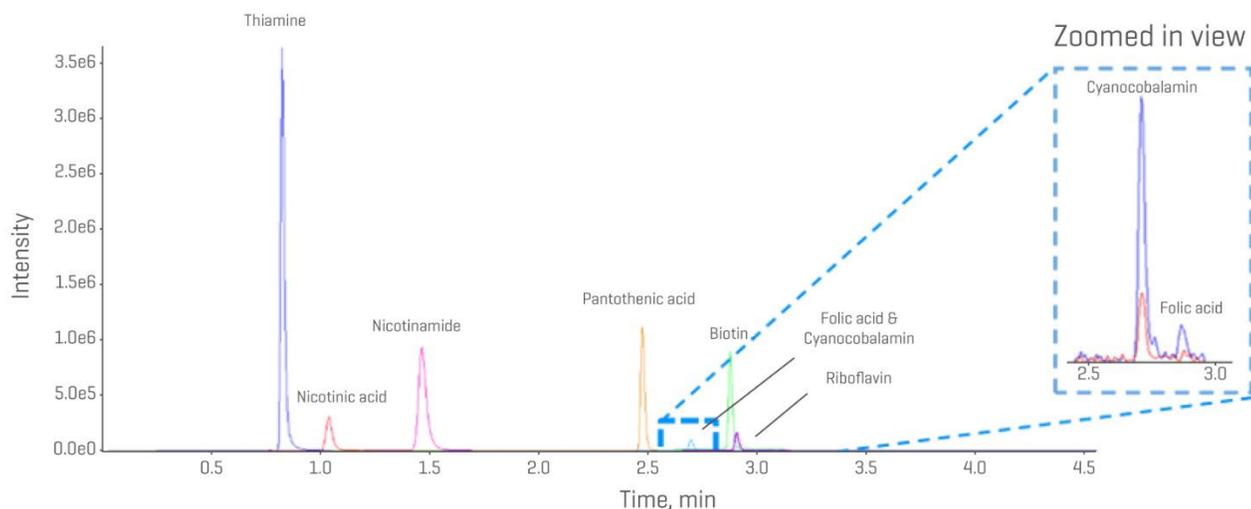
Figure 1. Chromatogram of 8 water-soluble vitamins extracted from serum matrix. Chromatogram of calibration standard for the water-soluble vitamins using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

Introduction

Water-soluble vitamins are essential for numerous metabolic processes and overall health. More specifically, B-complex vitamins, including B1 (thiamine), B2 (riboflavin), B3 (niacin), B9 (folate), and B12 (cobalamin), are vital for energy production, red blood cell formation, and proper nervous system function. Accurate measurement of these vitamins in biological samples is critical for assessing deficiencies.

Methods

Sample preparation: Water-soluble vitamins were extracted from human serum using a protein precipitation procedure with cold acetonitrile, evaporated to dryness under nitrogen, and reconstituted in water.

Liquid chromatography: Chromatographic separation was achieved using a Phenomenex Kinetex Luna Omega Polar C18 column (100 x 2.1 mm, 1.6 µm, 00D-4748-AN). Mobile phase A was ammonium formate in water with 0.1% (v/v) formic acid, and mobile phase B was methanol. The LC flow rate was 700 µL/min, and the total run time was 5 minutes. The injection volume was 10 µL.

Mass spectrometry: Data was collected using a QTRAP 6500+ system with an IonDrive Turbo V source and operated in electrospray ionization (ESI) positive mode. The scheduled MRM algorithm was used in SCIEX OS software (version 3.1.6) to collect 10-12 data points for quantifiable data. Compound-dependent parameters were optimized by infusion.

Data processing: Data processing was performed using SCIEX OS software (version 3.1.6). Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations and assay precision statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of 8 water-soluble vitamins in a control human serum sample. The 5 min gradient, in combination with the column selection and mobile phase composition, resulted in baseline separation of the 8 water-soluble vitamins. The extracted ion chromatograms showed S/N of 207:1, 87:1, 69:1, 69:1, 28:1, 118:1, 9:1, 11:1, and 16:1 for Thiamine, Riboflavin, Nicotinamide, Nicotinic acid, Pantothenic acid, Biotin, Cyanocobalamin, and Folic acid, respectively, at the lowest matrix calibrator measured, calculated using a peak-to-peak algorithm.

The quantitative performance of the method was investigated by injecting a series of serum calibrator samples spiked at different concentrations (0.25-100 ng/mL for thiamine, 0.41-100 ng/mL for riboflavin, 0.07-102 ng/mL for nicotinamide, 0.1-100 ng/mL for nicotinic acid, 0.5-1000 ng/mL for pantothenic acid, 0.05-100 ng/mL for biotin, 10-1000 ng/mL for cyanocobalamin and 0.2-100 ng/mL for folic acid. Linearity and precision were assessed across the calibration ranges for each of the eight analytes. Figure 2 shows the calibration curves for 8 water-soluble vitamins over the analytes' respective calibration ranges. The plots show excellent linear responses across the calibration series, with r^2 values greater than 0.99 for analytes.

The precision values were calculated from 3 replicates of the lowest matrix calibrators analyzed. The precision (%CV) were 0.4% for thiamine, 1.6% for riboflavin, 2.7% for nicotinamide, 11.3% for nicotinic acid, 0.4% for pantothenic acid, 6.2% for biotin, 5.0% for cyanocobalamin and 1.7% for folic acid.

Conclusions

A fast and sensitive LC-MS/MS method for the detection of water-soluble vitamins extracted from human serum samples was developed. The method demonstrated:

- Fast sample preparation which consisted of a simple protein deproteination
- Chromatographic separation of 8 water-soluble vitamins
- Excellent linear responses across the calibration series, with r^2 values greater than 0.99 for both analytes
- High quantitation performance of the method, resulting in excellent precision at the lowest calibrator levels
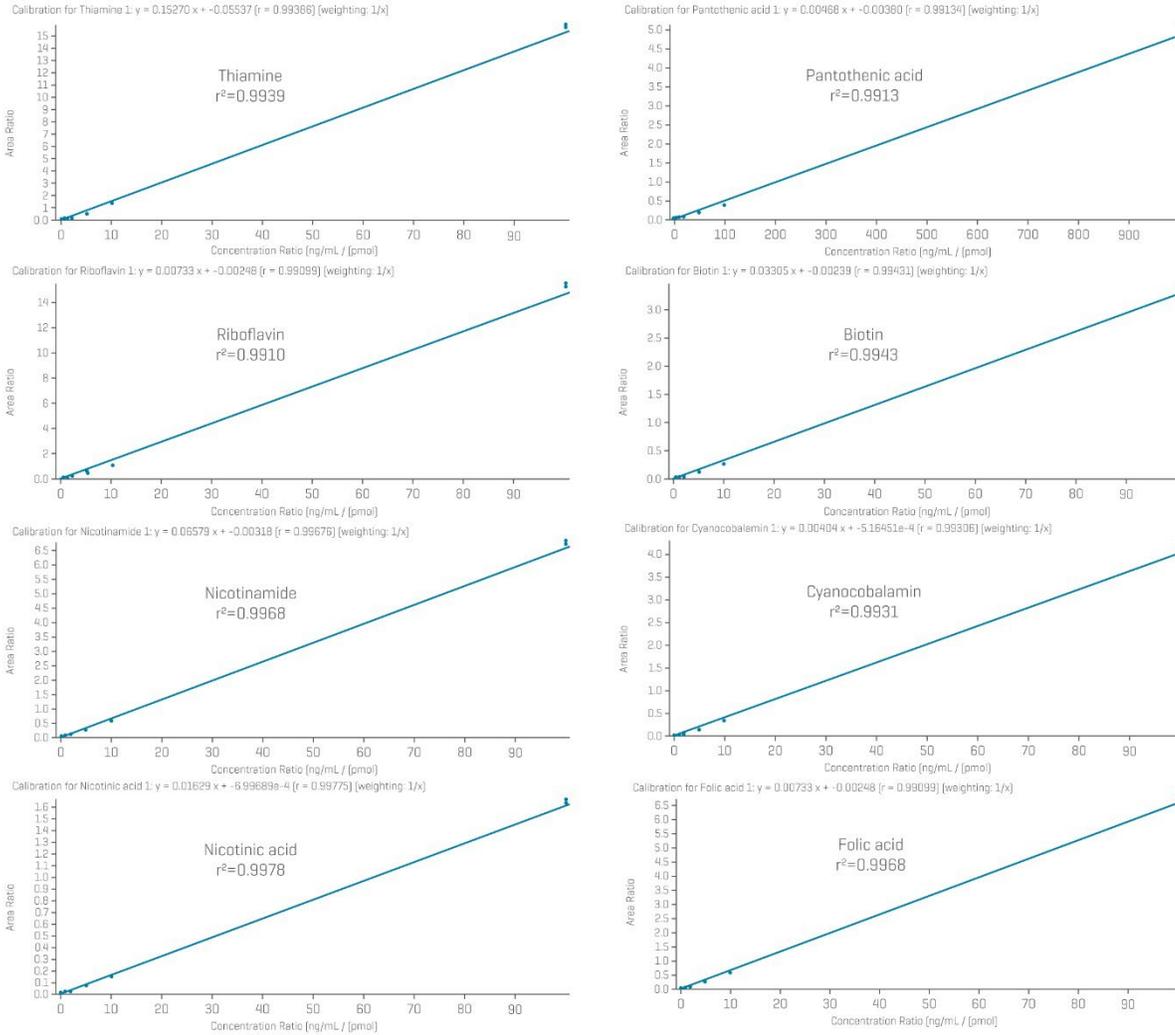
Figure 2. Linear calibration curves for the 8 water-soluble vitamins extracted from serum matrix. The calibration curves were run across the measured ranges. The curves were generated using linear regression and 1/x weighting for water-soluble vitamins in serum, resulting in r^2 values >0.99 for all the analytes.