Sensitive quantitation of vitamin B1 and B6 in human whole blood

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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+ system enabled accurate quantitation of vitamin B1 and B6 extracted from human whole blood. Excellent linearity was observed across clinically relevant concentrations. Low-nmol/L level sensitivity was achieved at the lowest calibrator with signal-to-noise ratios (S/N) of 769:1 for vitamin B1 at 39 nmol/L and 499:1 for vitamin B6 at 21 nmol/L. In addition, the method showed excellent precision and accuracy at low-level concentrations, demonstrating the quantitative performance of the assay.

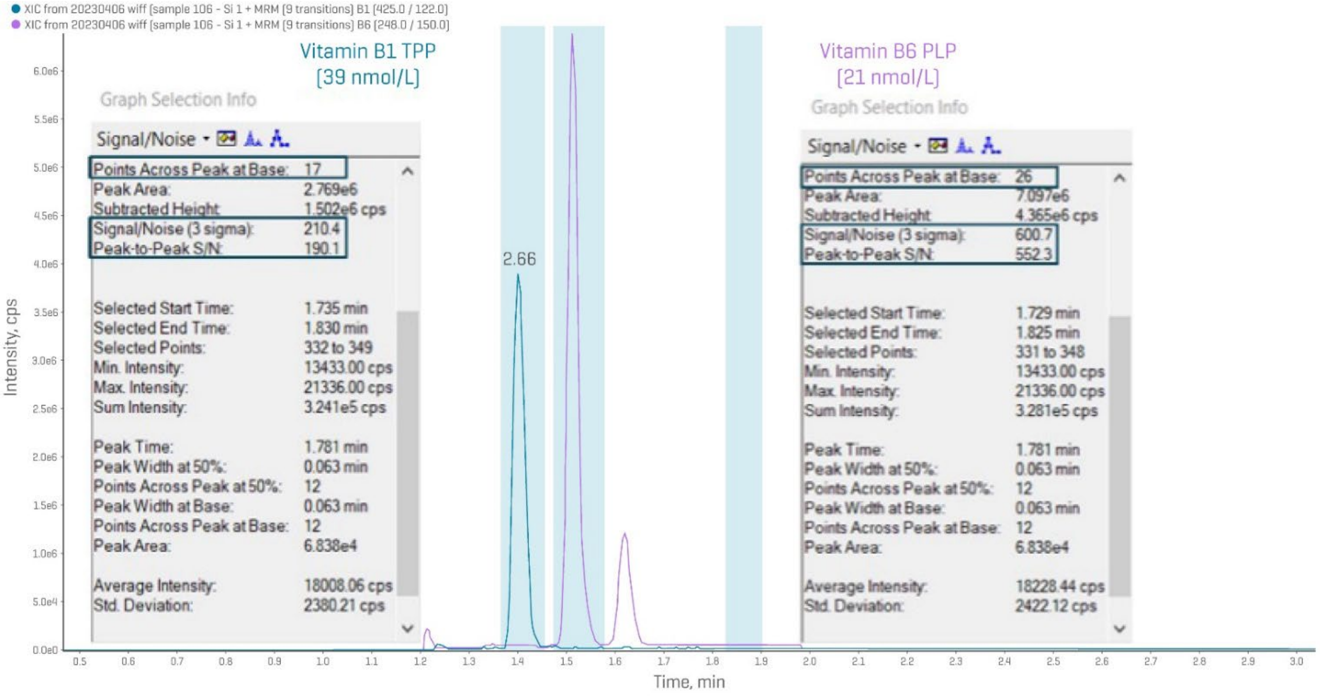
Key benefits of vitamin B1 and B6 analysis from human whole blood using the QTRAP 6500+ system

Low-nmol/L level sensitivity and excellent quantitative performance: Sensitive quantitation of vitamin B1 and vitamin B6 was performed with excellent precision (5.2% for vitamin B1 and 1.8% for vitamin B6) and accuracy 99.6% for vitamin B1 and 100.8% for vitamin B6 at the lowest calibrator levels (39 nmol/L for vitamin B1 and 21 nmol/L for vitamin B6)

Rapid sample preparation: Vitamin B complexes were extracted from human whole blood samples using a protein precipitation procedure

Chromatographic separation: Optimized LC conditions enabled fast (4 minutes) chromatographic separation of vitamin B1 and vitamin B6

Excellent linearity: Calibration curves for vitamin B1 and vitamin B6 showed r^2 values above 0.99 across the calibration range



Points Across Peak at Base:	17
Peak Area:	2.769e6
Subtracted Height	1.502e6 cps
Signal/Noise (3 sigma):	210.4
Peak-to-Peak S/N:	190.1
Selected Start Time:	1.735 min
Selected End Time:	1.830 min
Selected Points:	332 to 349
Min. Intensity:	13433.00 cps
Max. Intensity:	21336.00 cps
Sum Intensity:	3.241e5 cps
Peak Time:	1.781 min
Peak Width at 50%:	0.063 min
Points Across Peak at 50%:	12
Peak Width at Base:	0.063 min
Points Across Peak at Base:	12
Peak Area:	6.838e4
Average Intensity:	18008.06 cps
Std. Deviation:	2380.21 cps

Points Across Peak at Base:	26
Peak Area:	7.097e6
Subtracted Height	4.365e6 cps
Signal/Noise (3 sigma):	600.7
Peak-to-Peak S/N:	552.3
Selected Start Time:	1.729 min
Selected End Time:	1.825 min
Selected Points:	331 to 348
Min. Intensity:	13433.00 cps
Max. Intensity:	21336.00 cps
Sum Intensity:	3.281e5 cps
Peak Time:	1.781 min
Peak Width at 50%:	0.063 min
Points Across Peak at 50%:	12
Peak Width at Base:	0.063 min
Points Across Peak at Base:	12
Peak Area:	6.838e4
Average Intensity:	18228.44 cps
Std. Deviation:	2422.12 cps

Figure 1. Chromatogram of vitamin B1 (blue) and vitamin B6 (pink) extracted from blood matrix. Chromatogram of calibration standards in matrix for vitamin B1 TPP at 39.0 nmol/L and vitamin B6 PLP at 21 nmol/L shows a S/N of 769:1 for vitamin B1 and 499:1 for vitamin B6 based on a peak-to-peak algorithm.

Introduction

B vitamins play an important role in energy production, brain function, and red blood cell formation. More specifically, neurotropic vitamins such as vitamin B1 (thiamine-diphosphate) and vitamin B6 (pyridoxine-5' phosphate) are micronutrients that are crucial for energy production to support nerve function and overall brain health. As a result, accurate quantitation of these vitamin complexes is important.

Methods

Sample preparation: Vitamin B1 and vitamin B6 were extracted from human whole blood using a protein precipitation procedure. In short, 50 µL of blank human whole blood spiked with vitamin B1 and vitamin B6 at six concentration levels were added to a centrifuge tube to which 50 µL deuterated vitamin B1 and vitamin B6 internal standards were added400 µL of a 12% (v/v) trichloroacetic acid deproteinization solution was added to the tube and vortexed for 30 seconds. The tube was immediately shaken for 30 minutes and centrifuged for 5 minutes at 10,000 x g. 200 µL of the supernatant was transferred to a vial for analysis.

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 water and mobile phase B was methanol. The LC flow rate was 600 µL/min and the total run time was 5 minutes. The injection volume was 20 µL. The LC gradient program is presented in **Table 1.**

Table 1: Chromatographic gradient for the analysis of vitamin B1 and B6 in human blood.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	48	52
1.0	48	52
1.5	3	97
2.5	3	97
2.51	96.5	3.5
5.0	96.5	3.5

Mass spectrometry: Data was collected using a QTRAP 6500+ system with an IonDrive Turbo V ion 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 the appropriate amount of data points for quantifiable data. Source and gas conditions are presented in **Table 2**. Compound-dependent parameters were optimized by infusion.

Table 2: Source and gas parameters for the analysis of vitamin B1 and B6 in human blood using the QTRAP 6500+ system.

Parameter	Value
Polarity	Positive
Ion source gas 1	60 psi
Ion source gas 2	70 psi
Curtain gas	30 psi
Source temperature	650 °C
Ion spray voltage	5200 V
CAD gas	High

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, assay precision, and accuracy statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of vitamin B1 and vitamin B6 into a control human whole blood sample at final concentration of 39.0 nmol/L for vitamin B1 and 21.0 nmol/L for vitamin B6, respectively. The 4 min gradient, in combination with the column selection and mobile phase composition, resulted in baseline separation of vitamin B1 and vitamin B6. The extracted ion chromatograms showed a S/N of 769:1 for vitamin B1 and 499:1 for vitamin B6, at the lowest matrix calibrator measured (39.0 nmol/L for vitamin B1 and 21.0 nmol/L for vitamin B6), calculated using a peak-to-peak algorithm.

Figure 2 shows the representative extracted ion chromatograms (XICs) for A) vitamin B1 and B) vitamin B6 across their respective concentration ranges (39.0-657.0 nmol/L for vitamin B1 and 21.0-361.0 nmol/L for vitamin B6).

The quantitative performance of the method was investigated by injecting a series of calibrator samples spiked at concentrations ranging from 39.0 to 657.0 nmol/L for vitamin B1 and 21.0 to 361.0 nmol/L for vitamin B6, respectively. Linearity, accuracy and precision were assessed across the calibration ranges for each of the two analytes. **Figure 3** shows

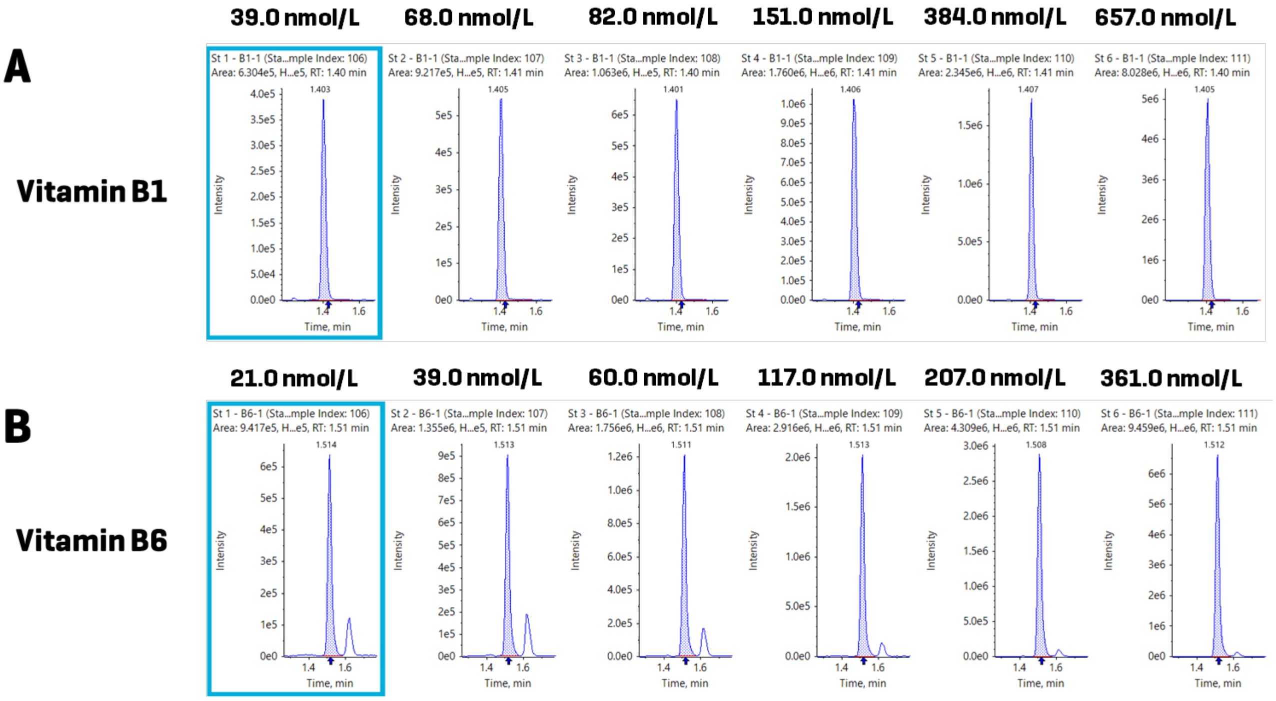


Figure 2. Extracted ion chromatograms for the two analytes targeted in this method. The XICs show the signal for A) vitamin B1 and B) vitamin B6 across their respective concentration ranges (39.0-657.0 nmol/L for vitamin B1 and 21.0-361.0 nmol/L for vitamin B6).

the calibration curves for vitamin B1 (left) and vitamin B6 (right) 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 both analytes.

The accuracy and precision values were calculated by 3 replicates of the lowest matrix calibrators analyzed (39.0 nmol/L for vitamin B1 and 21.0 nmol/L for vitamin B6). The accuracy was 99.6% for vitamin B1 and 100.8% for vitamin B6, respectively. The precision (%CV) was 5.2% for vitamin B1 and 1.8% for vitamin B6, respectively.

Conclusions

A fast and sensitive LC-MS/MS method for the detection of vitamin B1 and B6 extracted from human whole blood samples was developed. The method demonstrated:

- Fast sample preparation which consisted of a simple protein deproteination, requiring 50 µL of human whole blood sample
- Chromatographic separation of vitamin B1 and vitamin B6
- Excellent linear responses across the calibration series consisting of 6 calibrators, with r^2 values greater than 0.99 for both analytes
- High quantitation performance of the method, resulting in excellent precision (5.2% for vitamin B1 and 1.8% for vitamin B6) and accuracy (99.6% for vitamin B1 and 100.8% for vitamin B6) at the lowest calibrator levels 39 nmol/L for vitamin B1 and 21 nmol/L for vitamin B6)

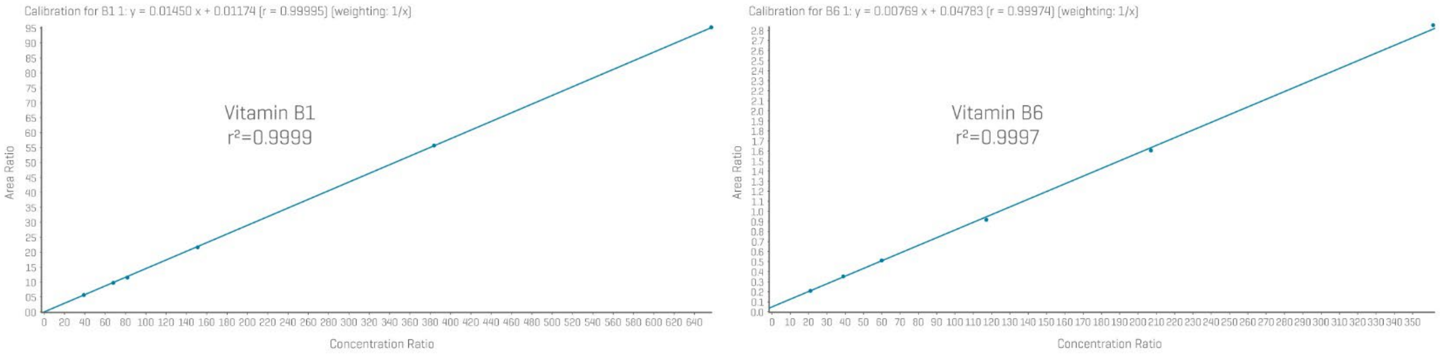


Figure 3. Linear calibration curves for vitamin B1 (left) and vitamin B6 (right) extracted from the blood matrix. The calibration curves were run across the following concentration ranges (39.0-657.0 nmol/L for vitamin B1 and 21.0-361.0 nmol/L for vitamin B6). The curves were generated using linear regression and 1/x weighting for vitamin B1 and vitamin B6 in blood, resulting in r^2 values of 0.9999 for vitamin B1 and 0.9997 for vitamin B6, respectively.

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