Analysis of the Vitamin B Complex in Infant Formula Samples by LC-MS/MS

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OVERVIEW
A capital sensitive and specific LC-MS/MS assay using the SCIEX QTRAP® 6500 system has been developed for the simultaneous detection of all major forms of vitamin B complex. The method detects all currently available vitamins and can be optimized further for specific laboratories, with internal standard preparation allowing for the sample to be used for both Vitamin C detection and the LC-MS/MS conditions have been formulated for each vitamin to be seen over the required dynamic range.

INTRODUCTION
The vitamin B complex is a group of water-soluble vitamins that play important roles in cell metabolism. The absence of individual B vitamins can lead to several conditions, including depression and high blood cholesterol. No general recommendation is available for the intake of the vitamin B complex, and it is understandable, for example, from the US Food and Drug Administration (USFDA) context, that infant formula is an important source of vitamins B6, B7, B9 and B12. Early eluting vitamins such as vitamin B3 are difficult to detect with the SCIEX QTRAP® 6500 system and require mild treatment, with SPExolonger needed for the late eluting B7, B9, and B12 vitamins.

Sample Preparation
Sampling 
A sample of infant formula was mixed with 50% acetonitrile in acidified water (containing an antioxidant) and internal standards. The sample preparation was kept as simple as possible to reduce vitamin degradation.

EXPERIMENTAL
Sample Handling
Samples (1 g) were weighed with 95% accuracy in a well-covered mini-bottle containing an antioxidant and internal standards solution was added. The mass sample solution was homogenized using a small glass homogenizer and left to cool for 10 minutes in an ice-cold bath. The pH of the solution was brought to the range of 2.0–2.5 using 50% acetonitrile in water.

Method Development
LC Separation
The samples were analyzed by LC on a packed microbore RP column using a SCIEX QTRAP® 6500 system, with an ion-pairing reagent. The conditions are shown in Table 1. The USFDA guidelines for baby foods require that the sample preparation be kept as simple as possible to reduce vitamin degradation.

LC-MS/MS Analysis
An SCIEX QTRAP® 6500 instrument and MultiQuant™ software version 3.0.2 were used for all experiments. A MRM™ algorithm was used to acquire data for a total of 26 transitions to ensure the best reproducibility and sensitivity. All results were processed in Prodigy™ software using version 2.2.0. The LC-MS/MS method utilized a small particle size packed microbore reversed-phase (RP) column and a 11 mm gradient. Very little sample preparation has been used to ensure high throughput for routine food testing.

RESULTS
The method’s dynamic range requirements and the required differences in detection required for the class of vitamins, carry response tests to be adjusted in order to maintain a linear response across the required range. The method’s performance was evaluated with linearity testing using the CE. The CE range was automatically generated during method development using the Compound Dependent Tandem Mass Spectrometry (CDTMS) assay application software. An example of this is shown in Figure 3. All results for the samples are summarized in Table 4.

SUMMARY
An LC-MS/MS method has been developed to detect the vitamin B complex in infant formula. Detection limits and lower concentration levels were also required to ensure that only the most sensitive B vitamins were detected. Additionally, the USFDA guidelines for infant formula require that the sample preparation be kept as simple as possible to reduce vitamin degradation.

REFERENCES
TRADEMARKS/LICENSES
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