Background on Vitamin B12

• Recognized as a fatal disease over 100 years ago, vitamin B12 deficiency causes megaloblastic anemia as well as neurological abnormalities.

• Development of effective dietary supplement therapy for “pernicious” anemia resulted in a Nobel Prize for Minot, Murphy and Whipple in 1934.

• Dorothy Hodgkin received a Nobel Prize in 1964 for her X-ray crystallographic structure determination of vitamin B12.
Background on Vitamin B12

- Vitamin B12 (cobalamin) is a group water soluble corrinoids with a cobalt-coordinated nucleotide containing the base, 5,6-dimethylbenzimidazole.
- Vitamin B12 is synthesized only in certain bacteria and becomes concentrated in higher organisms along the food chain.
- Therefore, animal-based foods are the primary sources of vitamin B12 in the human diet.
- Vegans and people with digestive insufficiencies are at greatest risk of vitamin B12 deficiency.

Vitamin B12 and Related Cobalamins

- 5'-Deoxyadenosylcobalamin and methylcobalamin are physiologically active.
- Often used in dietary supplements, other cobalamins can be converted in vivo.
- Corrinoids containing bases other than 5,6-dimethylbenzimidazole are inactive.
General Analytical Needs

- Method should
  - measure the physiologically active vitamin B12 compounds
    - 5'-deoxyadenosylcobalamin and methylcobalamin
  - measure the provitamin B12 forms
    - Including hydroxocobalamin, sulfocobalamin and cyanocobalamin (which is the form most often used in dietary supplements)
  - distinguish between vitamin B12 active corrinoids containing the base, 5,6-dimethylbenzimidazole and inactive forms present in some dietary supplements (especially those derived from edible cyanobacteria).

Analytical Challenges

- Quantitatively extract vitamin B12 compounds from a variety of matrices including finished products such as capsules and pills and unprocessed raw materials such as cyanobacteria.
- Measurement of trace levels of vitamin B12 compounds in natural sources as well as in fortified samples.
- Measure multiple vitamin B12 compounds individually or after derivatization to a common form such as cyanocobalamin.
- Distinguish vitamin B12 cobalamins from inactive forms.
Regulations

• Intake recommendations for vitamin B12 are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences).
• For healthy adult men and women (not pregnant or lactating), the recommended daily allowance is 2.4 μg.
• RDAs for other ages: 0–6 mos 0.4 μg; 7–12 mos 0.5 μg; 1–3 yr 0.9 μg; 4–8 yr 1.2 μg; 9–13 yr 1.8 μg; 14+ yr 2.4 μg
• Prescription injectable (im), intranasal and parenteral forms of vitamin B12 are available.

Current Analytical Methods for Vitamin B12

• Bioassay using vitamin B12 dependent bacteria, such as Lactobacillus delbrueckii subsp. lactis ATCC7830
• Radioimmunoassay (RIA) and radioisotope dilution assays using radioactive ⁵⁷Co & binding protein (intrinsic factor)
• Chemiluminescence using acridinium ester-labeled vitamin B12 and intrinsic factor
• Surface plasmon resonance of prepared samples
• HPLC-UV following immunoaffinity extraction
• HPLC-UV following solid phase extraction, with or without derivatization (conversion) to cyanocobalamin
• HPLC-MS and HPLC-MS/MS
Existing Methods for Vitamin B12

- USP – cyanocobalamin and hydroxocobalamin pure substances and injectable solutions, tablets and capsules by spectrophotometry and HPLC-UV
- AOAC International
  - 952.20 vitamin preparations by microbiological assay
  - 986.23 milk-based infant formula by microbiological assay
  - 2011.08 and 2011.09 infant formula and adult nutritionals by HPLC-UV with immunoaffinity extraction after conversion to cyanocobalamin (first action)
  - 2011.10 infant formula and adult nutritionals by HPLC-UV with column switching after solid phase extraction
  - 2011.16 infant formula and adult nutritionals by surface plasmon resonance

Fitness for Purpose (proposal)

The method for vitamin B12 dietary supplement analysis must quantitate multiple forms of vitamin B12 individually or after conversion to a common form (such as the more stable cyanocobalamin) in a variety of dosage forms. The method must also be able to distinguish between active vitamin B12 corrinoids and inactive forms present in products derived from some microbiological sources. As humans can only absorb 10 to 500 μg B12/day and the RDA is from 0.4 to 2.8 μg B12/day, the analytical range for supplements should extend from at least 0.1 to 1000 ppm per dosage unit.
QUESTIONS?