# Determination of Vitamin $B_{12}$ in Multivitamins using HPLC-ICP-MS

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## 1. Introduction

Vitamin $B_{12}$ (cobalamin) plays a vital role in red blood cell formation and in the regular physiology of the nervous system. Naturally occurring cobalamins are only found in animal products such as meat, eggs, and dairy. Dietary supplements such as multivitamins can be used to increase nutrient intake. Traditional methods for vitamin $B_{12}$ determination include microbiological assays, spectrophotometric, and various chromatography techniques, many of which are limited in sensitivity, specificity, and efficiency. Inductively coupled plasma-mass spectrometry (ICP-MS) is an alternative technique used to determine the content of vitamin $B_{12}$ indirectly by measuring the complexed cobalt in cobalamins. This poster presents the development of a method for vitamin $B_{12}$ determination in multivitamins based on sample extraction followed by analyzing the filtrate by high performance liquid chromatography (HPLC) interfaced with ICP-MS.

## 2. Experimental

***Note: All experiments were carried out with subdued lighting and amber vials to avoid photolytic degradation of the analytes.***

### 2.1. Instrumentation, standards and SRMs

- A shaking water bath from Boekel Scientific was used for extraction. Analysis was performed using Agilent 1260 HPLC coupled with an Agilent 7900 ICP-MS.
- Standards of cyano-, hydroxo-, adenosyl- and methylcobalamin (CNCbl, OHCbl, AdoCbl and $CH_3$Cbl), and pepsin and α-amylase enzymes were purchased from Sigma-Aldrich.
- Standard reference materials (SRM) of multivitamins from NIST (3280) and NRC Canada (VIT-A1 and VIT-B1) were used.

***Table 1.*** *Chromatographic conditions for separation of cobalamin species.*

| Column | Mobile phase(s) | Gradient |
|---|---|---|
| Zobrax Eclipse XDB-C8 (3 x 150 mm, 3.5 µm) | 0.1% formic acid in (A) water and (B) methanol | 0–15.0 min (5–50% B), 15.1–16.0 (50% B), 16.1–25.0 min (0%B) |

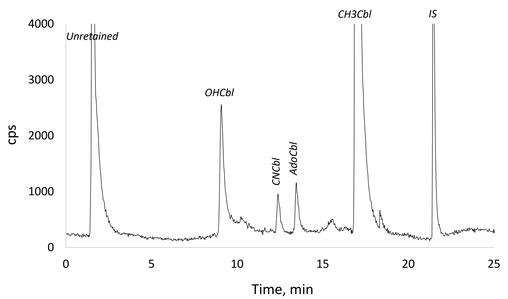


***Fig. 1.*** *Chromatogram showing the separation of cobalamin species extracted from a multivitamin capsule. IS - post-column injected standard.*

### 2.2. Sample homogenization

- Fifteen tablets, chewables, nuggets or lozenges of a multivitamin or the contents of 15 capsules were finely ground with IKA tube mill. For powder multivitamins, 15 serving sizes were combined and ground, and liquid samples were analyzed as is.
- The homogenized sample was transferred to amber centrifuge tube and stored at 4°C.

## 3. Results and Discussion

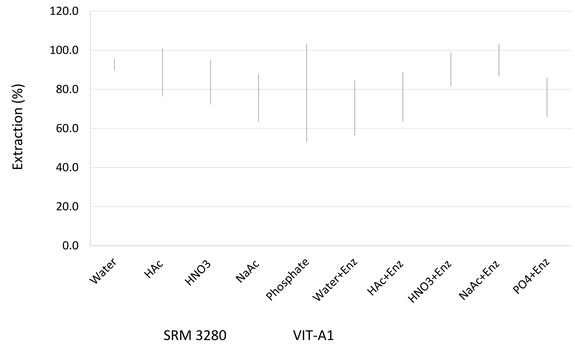


***Fig. 2.*** *Cobalamin extraction (%) form three SRMs using different extraction conditions.*

### 3.1. Extraction of cobalamin species

- Several extraction solutions (Table 2) were tested with and without enzymes to extract cobalamins from multivitamins. The enzymes were pepsin (0.5 g) and α-amylase (300 µL) in 10 mL solution.
- Half-a-gram of an SRM was weighed out into amber centrifuge tube, mixed with 10 mL of the extractant and heated to 40°C in a water bath for 2 hours. The extract was filtered and analyzed by HPLC-ICP-MS (Table 1).
- Fig. 2 shows the recovery of cobalamin from the three SRMs. The length of the vertical lines in the figure indicate the range of the cobalamin extraction recovery for the three SRMs.

***Table 2.*** *Solutions evaluated for extraction of cobalamins from multivitamins.*

| Solvent/solution | Concentration, pH |
|---|---|
| Water | - |
| Acetic acid | 1% (v/v) |
| Nitric acid | 1% (v/v) |
| Sodium acetate | 0.25 M, pH 4.5 |
| Phosphate buffer | 0.05 M, pH 5.8 |

- The above conditions were also tested to extract cobalamins from three multivitamin tablets.
- It can be seen from Fig. 3 that sodium acetate with enzymes gave relatively better extraction from the three samples. Considering its better performance, this extractant was selected for routine analysis of multivitamins.

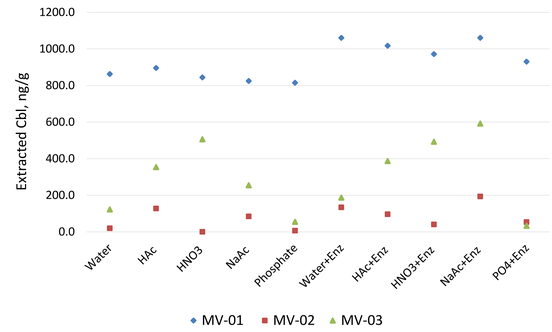


***Fig. 3.*** *Cobalamin (ng/g) extracted from three multivitamin samples using different extraction conditions.*

### 3.2. Stability of cobalamin species

- Separate standard solutions (0.23 µg/g) of CNCbl, OHCbl, $CH_3$Cbl and AdoCbl were extracted (in duplicate) with enzymatic solution of sodium acetate as described in Section 3.1.
- After filtering into amber vials, one replicate of each extract was kept in a dark room at ambient temperature and the other was preserved at 4°C.
- Fig. 4 shows the fractions of each cobalamin species recovered 1, 2, 3 and 7 days after extraction. While CNCbl stayed stable throughout in both environments, the other species lack stability from the beginning. However, keeping the cobalamins at 4°C helps to slightly increase their lifetimes.
- It was observed that $CH_3$Cbl and AdoCbl were converted to OHCbl, but the transformation product of OHCbl could not be identified in the chromatograms.

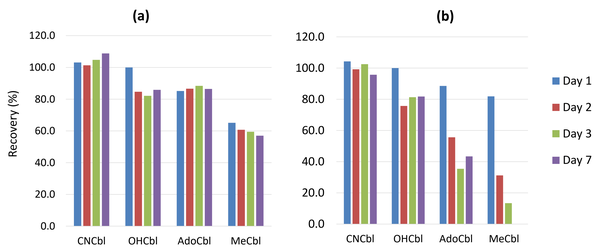


***Fig. 4.*** *Stability of cobalamin species in standard solutions after extraction. Extracts were (a) refrigerated at 4°C or (b) kept in a dark room at ambient temperature.*

### 3.3. Analysis of Samples

- Multivitamins in the forms of tablet, capsule, chewable, nugget, lozenge, liquid and powder with declared levels and forms of cobalamins were analyzed following the procedure described in described in Section 3.2.
- Due to the lack of stability exhibited by OHCbl, $CH_3$Cbl and AdoCbl, the concentrations of the cobalamin species were determined based on external calibration constructed using CNCbl.
- Fig. 5 compares the measured concentrations of the cobalamin species with their declared values.
- The measured concentrations are either above or close to the claimed concentrations for the samples except the two products that contain OHCbl. The reason for the latter needs further studies.
- Table 3 lists other cobalamins than the claimed forms found in the tested samples. OHCbl and AdoCbl were found in some of the products at relatively lower concentrations compared to the level of the main cobalamin form in the respective products.

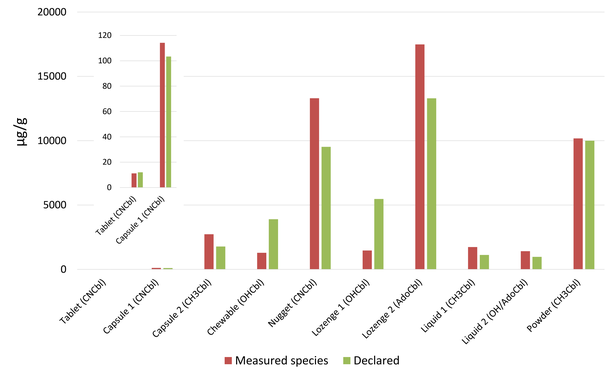


***Fig 5.*** *Cobalamin species measured in the multivitamin samples compared against their declared concentrations.*

***Table 3.*** *Cobalamin species other than the claimed forms found in the multivitamin samples.*

| Sample | Claimed Species | Other Species Found, µg/g | | | |
|---|---|---|---|---|---|
| | | ***OHCbl*** | ***CNCbl*** | ***AdoCbl*** | ***$CH_3$Cbl*** |
| Tablet | CNCbl | | | | |
| Capsule 1 | CNCbl | | | | |
| Capsule 2 | $CH_3$Cbl | 244 | 41 | 70 | |
| Chewable | OHCbl | | | 150 | |
| Nugget | CNCbl | | | | |
| Lozenge 1 | OHCbl | | | 290 | |
| Lozenge 2 | AdoCbl | 324 | | | |
| Liquid 1 | $CH_3$Cbl | 83 | | | |
| Liquid 2 | OH/AdoCbl | | 53 | | 36 |
| Powder | $CH_3$Cbl | 398 | | 229 | |

## 4. Conclusion

- A method has been developed which offers adequate solution to determine cobalamin species in various forms of multivitamins. The potential application of the method to other nutritional products will be evaluated.

## 5. Reference

1. Qui et al., Determination of active vitamin $B_{12}$ (cobalamin) in dietary supplements and ingredients by reversed-phase liquid chromatography: Single-laboratory validation, Food Chem 298 (2019) 125010.