

**Single Lab Validation of a Two-column LC/UV/Vis Method for Determination of
Cyanocobalamin in a Multivitamin Dietary Supplement**

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A HPLC-UV/VIS method using a two-column strategy with a switching valve for on-line sample clean-up was developed for the determination of cyanocobalamin (CN-Cbl/Vitamin B₁₂) in dietary supplements. The method uses two columns, an Agilent Zorbax - C8 (150 mm × 4.6 mm, 5 μm particle size) reversed-phase (RP) column and a Waters Symmetry C18 (150 mm × 4.6 mm, 5 μm particle size) RP column. Chromatographic separation was achieved using a programmed gradient mobile phase consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The recovery of CN-Cbl was 99.5% and the limit of quantitation (LOQ) was 10 ng per injection. The method was successfully applied to the analysis of the NIST SRM3280 dietary supplement. The method is specific, precise, and accurate for the intended use. Compare to off-line sample clean-up procedures, it offers the advantage of being easier, more economical, and less time-consuming.