

Application note

Extraction of vitamin B₁₂ from multivitamin tablets

Field	Pharmaceutical/Clinical
Sample	Vitamin B ₁₂ (Cobalamin)
Matrix	Multivitamin Tablets
Extraction Column	BAKERBOND spe™ Quaternary Amine (N+), 3 mL (500 mg); Phenyl (C ₆ H ₅), 3 mL (500 mg); Filtration Column, 3 mL
Safety and Protective Equipment	Goggles and face shield, lab coat and apron, vent hood, proper gloves, Type B fire extinguisher.
Sample Preparation	Add 10 mL extracting solution (1.3 g dibasic sodium phosphate, 1.2 g citric acid monohydrate, 1 g sodium metabisulfite/100 mL of HPLC grade water) to 1 tablet weight of multivitamin tablet powder in a 25 mL low actinic flask. Sonicate for 2 minutes. Shake on a mechanical shaker for an additional 15 minutes. Allow flask to stand undisturbed for 2 minutes before sampling.
Standard Preparation	Dissolve appropriate amount of B ₁₂ standard in a given volume of extracting solution. Make proper dilutions with extracting solution to give the B ₁₂ concentration expected in the sample solution. Treat standard as if it were a sample for the remainder of the procedure.
Column Conditioning	Condition both the quaternary amine and phenyl columns with 3 mL methanol, followed by 3 mL HPLC grade water, followed by 2 x 3 mL extracting solution. With vacuum off, fill each column with extracting solution. Place adapter on top of each column and fit the quaternary amine column into the adapter on top of the phenyl column. Attach a 6 mL filtration column to the adapter on top of the quaternary amine column.
Sample Addition/Wash	Transfer 2 mL of sample solution to the filtration column and aspirate solution through entire column assembly. Wash columns with 2 x 1 mL of extracting solution and remove the filtration and quaternary amine columns. Wash the phenyl column with 1 mL HPLC grade water. Dry interior of column with a cotton swab (be sure to remove all water droplets) and air dry column under vacuum for 5 minutes. Wash the column with 3 mL hexane, followed by 1 mL methylene chloride, followed by 2 mL acetonitrile (dried over anhydrous sodium sulfate), followed by 2 mL acetonitrile/ methanol (dried over anhydrous sodium sulfate) (95:5). Do not allow columns to dry between solvent additions. Air dry column under vacuum for 1 minute after the acetonitrile/ methanol wash.
Sample Elution	Elute with 2 x 0.5 mL methanol/HPLC grade water (9:1) collecting the eluate in a 1 mL volumetric flask. Dilute to volume with the eluting solvent and mix well.

Product list

Description	Product Number
BAKERBOND spe™ Quaternary Amine (N+), 3 mL (500 mg)	JT70913
BAKERBOND spe™ Phenyl (C ₆ H ₅), 3 mL (500 mg)	JT70953
Filtration Column, 6 mL	JT71216
SPE Column Adaptor	JT7300-0
Acetonitrile, 'BAKER ANALYZED'® HPLC	JT9017-3
Citric Acid, Monohydrate, 'BAKER ANALYZED'® ACS Reagent	JT0110-1
Hexanes, 'BAKER ANALYZED'® HPLC	JT9304-3
Methanol, 'BAKER ANALYZED'® HPLC	JT9303-3
Methylene Chloride, 'BAKER ANALYZED'® HPLC	JT9315-3
Sodium Metabisulfite, 'BAKER ANALYZED'® ACS Reagent	JT3552-1
Sodium Phosphate, Dibasic, 'BAKER ANALYZED'® ACS Reagent	JT3828-1
Sodium Sulfate, Anhydrous, 'BAKER ANALYZED'® ACS Reagent	JT3891-1
Water, 'BAKER ANALYZED'® HPLC	JT4218-3