Application

(w) www.eprep-analytical.com
(e) info@eprep.com.au

ePrep ONE® | Application Note 2026

Automated Sample Preparation
Cyanocobalamin (Vit B12) for Assay and ID by UV-Vis

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Authors
Ego Pharmaceuticals:
H. Evans-Lemmo, L. Sheedy
METTLER TOLEDO:
C. Voskuilen
ePrep:
A.Minett

Keywords
- Cyanocobalamin Assay
- Cyanocobalamin ID
- Vitamin B12
- USP Monograph
- BP Monograph
- EP Monograph
- CFR Part 11
- Sustainability
- ePrep ONE
- METTLER TOLEDO UV7
- LabX

Application Benefits

- **Regulatory Compliance:** The ePrep ONE and METTLER TOLEDO UV7 UV-Vis Spectrophotometer with LabX™ ensure Cyanocobalamin sample preparation and analysis meets USP, EP and BP monograph compliance standards.
- **Operational Efficiency:** ePrep ONE in partnership with the METTLER TOLEDO UV7 UV-Vis Spectrophotometer with LabX automates the analysis workflow
- **Accelerate Scientific Innovation:** enabling scientists to focus on innovation and complete projects faster.
- **Sustainability:** ePrep ONE together with the METTLER TOLEDO TX Autotitrator with LabX supports green chemistry and sustainable lab operations without sacrificing performance.

Application Purpose

This application solves the inefficiency, high cost, and environmental burden of traditional manual Vitamin B12 (cyanocobalamin) extraction and analysis by replacing it with a fully automated, pharmacopeia-compliant workflow.

Summary

Vitamin B12 assay is a critical quality control measure. B12 is an expensive API susceptible to counterfeiting, adulteration, and supply chain fraud, making traceable analysis at the manufacturing site essential for ensuring quality and patient safety. This document describes the transition from traditional manual extraction and titration to an end-to-end automated process using the ePrep ONE Sample Extraction Workstation and METTLER TOLEDO UV7 Spectrophotometer with LabX™ Software, ensuring compliance to EP, BP, and USP monographs.

While the manual UV-Vis assay is not technically challenging, the specified 30 µg/mL concentration and reliance on volumetric glassware demands larger sample masses, more solvent, and significant analyst time. The ePrep ONE completes stock preparation and sample dilution without analyst intervention, and evaluation of precision, linearity, and robustness confirmed performance equivalent to or better than manual methods. The automated workflow delivers time, solvent, and cost savings, improved precision and accuracy, reduced environmental impact, and a 21 CFR Part 11 compliant validated process.

Key Objectives

The following objectives guided the development and validation of the automated B12 assay workflow:

- Develop an automated sample preparation for the assay method compliant with both BP and USP requirements.
- Validate the automated workflow
- Demonstrate equivalence in precision and accuracy to meet USP and BP compendia requirements
- Enhance efficiency by reducing time, solvent usage, and labour

Integrated Technology

The integration of two specialist technologies, METTLER TOLEDO for gravimetric and spectrophotometric measurement and ePrep's automated sample preparation system, combines purpose-built domain expertise that a single-vendor solution cannot achieve. This dual-vendor architecture ensures each component of the analytical workflow is governed by validated, best-in-class instrumentation optimised for its specific function.

The ePrep ONE is a syringe-based sample preparation system accommodating volumes from 10 µL to 10 mL, with positive displacement enabling reproducible dispensing to 1 µL. Stainless-steel needles mitigate solvent incompatibility and cross-contamination while eliminating single-use plastic consumables. The configurable software supports method development through predefined coordinate mapping and reusable workflow templates, facilitating preparation of cuvette-ready samples for UV-Vis spectrophotometric analysis, either as individual time-critical extractions or complete sample sets optimised for automated cuvette changer operation.

LabX software unifies METTLER TOLEDO instrumentation (Analytical Balance and UV7 Spectrophotometer) within a single platform. Combined with the 21 CFR Part 11 validated ePrep Axis software, complete digital traceability is established from initial gravimetric measurement through sample preparation to final spectrophotometric result, delivering end-to-end regulatory compliance across the entire analytical workflow.

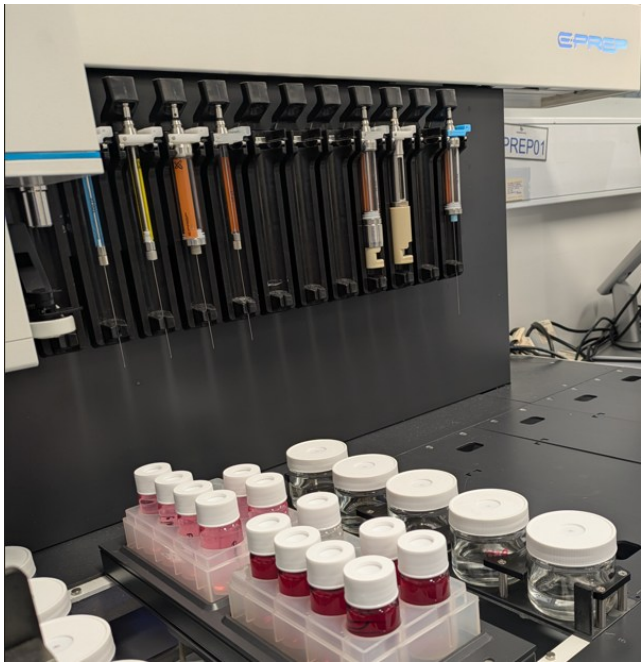

Figure 1: Automated Extraction of Vitamin B12 Identification and Assay using ePrep ONE

Full 21 CFR Part 11 Compliance

Both ePrep ONE and LabX software are validated to 21 CFR Part 11 requirements, providing a full audit trail and ensuring GMP compliance across the analytical workflow. Standardised programmed methods remove operator-dependent variability, mitigating the risk of Out of Specification /Out of Trend results, laboratory rework and associated costly investigations. Supporting reliable batch release decisions irrespective of individual analyst proficiency.

Experimental

All samples and standards were weighed as shown in Table 1, using an Analytical Balance from METTLER TOLEDO. They were then prepared using the fully automated sample extraction instrument, ePrep ONE. The equipment needed for performing sample extraction on ePrep ONE is listed in Table 2.

Table 1 Reagents and Solvents required for Assay of Cyanocobalamin

Name	Grade	Brand	Mass
Purified Water	RO	-	-
Sample for Analysis	BP	-	0.0400 ± 0.0040 g

Table 2 Equipment required for required for Assay of Cyanocobalamin using ePrep ONE and UV7

Glassware	Syringes/Tools	Decks
50 mL Glass Reagent Jars	10 mL Standard Syringe	2 x Reagent Jar Rack
20 mL Glass Vials	1 mL Standard Syringe	Vortex Mixer Rack

Following extraction of all Samples and Standards, analysis was performed by UV Spectroscopy, METTLER TOLEDO UV7 using the conditions specified in table 10.

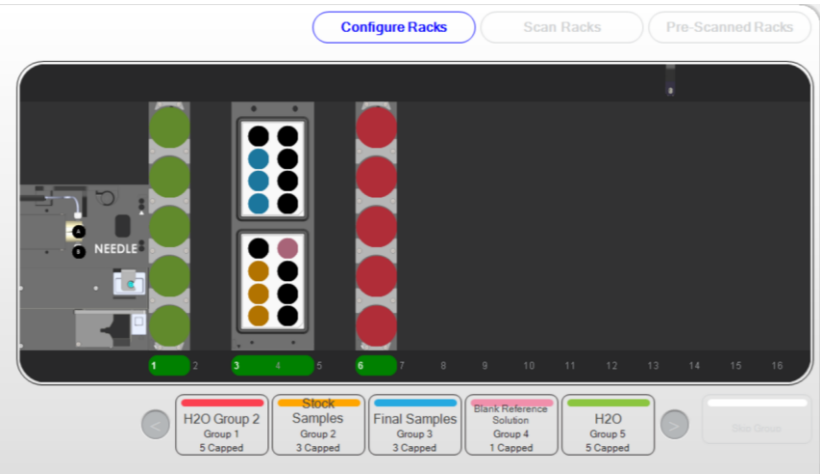

Figure 2 Screen capture of the ePrep ONE deck for Assay of Cyanocobalamin (3 samples + 1 blank)

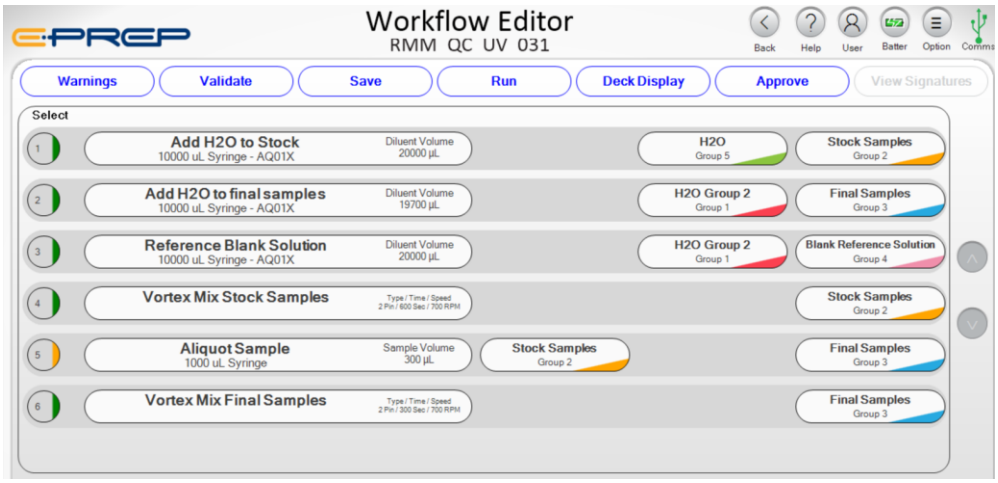

Figure 3 Screen capture of the ePrep ONE application for Assay of Cyanocobalamin

The deck setup required for the ePrep ONE workflow, and the defined groups are shown in Figure 2. The workflow Tasks used to complete the ePrep ONE extraction is given in Figure 3.

Comparison of BP and USP Monographs to ePrep ONE

The ePrep ONE automated extraction application is compared to the manual extraction described in the BP monograph. The BP and EP monographs specify a 25 µg/mL sample concentration, the USP a concentration of 30 µg/mL, the development team trialled both solution concentrations in development and found no significant difference, the USP concentration of 30 µg/mL was selected for the validation. The monograph methods specify dissolution of Cyanocobalamin in water, and diluted to the working sample concentration. The resultant solutions are analysed at 361nm using a blank of water.

Automated Sample Preparation

In the automated ePrep ONE application, the sample identification is scanned using LabX. 0.0400 g of the sample for analysis is weighed into a 20 mL vial and placed by the Analyst onto the ePrep ONE instrument group 2, deck position 3 front, yellow Figure 2. A 20 mL vial for the blank is also placed onto the deck, group 4, deck position 3 front, pink Figure 2. Capped 20 mL vials are placed onto the deck for the final samples, group 3, deck position 3 back, blue Figure 2.

The ePrep ONE sample preparation uses the 10 mL syringe to add 20 mL of water to the stock samples. The solution is then vortex mixed by the ePrep ONE for 10 minutes at 700 RPM.

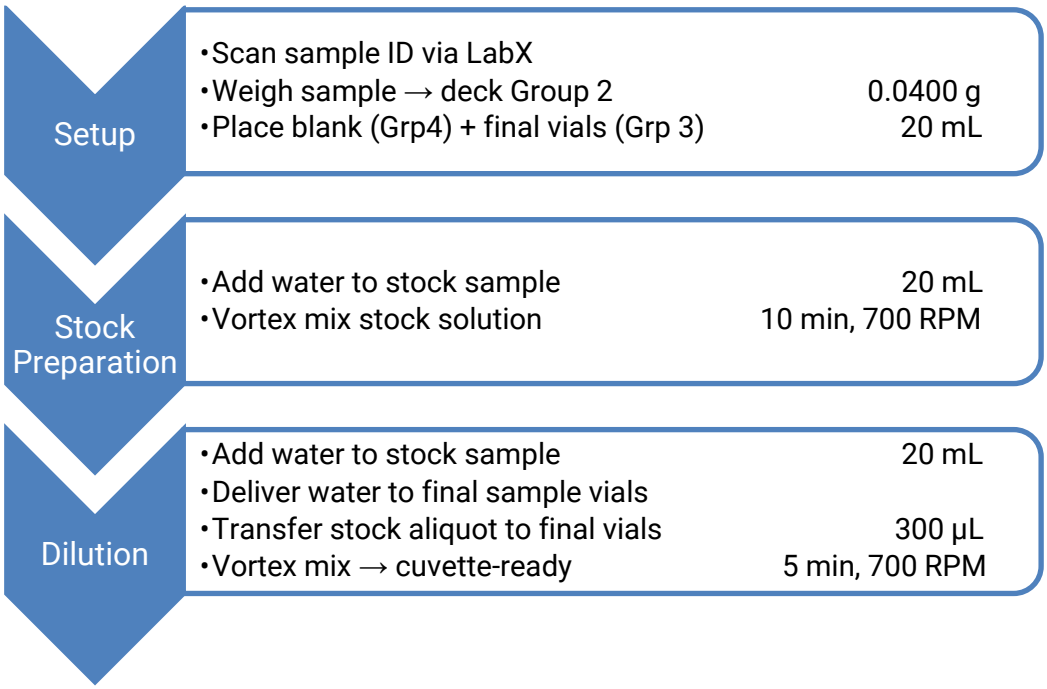

Figure 4 Simplified flow diagram of the ePrep ONE application for Assay of Cyanocobalamin

A 10 mL syringe is used to add 20 mL of water to the blank vial, group 4, deck position 3 front, pink. The same 10 mL syringe is used to deliver water to the final sample vials, group 3, deck position 3 rear, blue. An aliquot of 300 µL of the stock sample is transferred to the final sample vials before being vortex mixed by the ePrep ONE for 5 minutes at 700 RPM.

ePrep ONE System Parameters

The following Parameters will be discussed in detail; however, a summary of the selected parameters are in Table 3.

Table 3 Summary Table for Parameters of ePrep ONE Sample extraction

Parameter	Chosen Option
Vial Choice	50 mL Glass Vials
	20 mL Glass Vials
Syringe & Needle Choice	10 mL ePrep ONE Syringe, Standard Needle
	1 mL ePrep ONE Syringe, Standard Needle
Aspiration Speed (µL/Sec)	175
Aspirate Pause (Sec)	3
Dispense Flow Rate (µL/Sec)	500
Dispense Pause (Sec)	3
Waste	ePrep ONE wash station
Mixing Type	Vortex Mixing

- Vial & Needle Selection: RFID-verified syringes with 20 mL and 50 mL glass vials ensure correct apparatus and accurate collection under Part 11 compliance.
- Aspiration & Dispense: 175 µL/sec aspiration minimises variability; 500 µL/sec dispense with 3 s pause and Auto Low needle depth prevents droplet carryover.
- Rinse Settings: Optimised rinse cycles eliminate inter-sample carryover.
- Waste & Wash: Automated syringe wash pre- and post-extraction maintains instrument readiness.

Results and Discussion

The ePrep ONE sample preparation was validated with respect to precision (repeatability), linearity, intermediate precision, and robustness. The following parameters were used to assess the transfer of sample preparation from manual extraction to automated extraction using ePrep ONE:

Precision (Repeatability):

Precision was evaluated by six replicate extractions from the same sample using ePrep ONE. Results yielded a %RSD of 1.06% (Table 4). All results were within the predefined specification limits, confirming that the automated ePrep ONE extraction method is precise.

Table 4 Precision using ePrep ONE.

Preparation	Result (% Cyanocobalamin)
1	96.0
2	98.9
3	98.3
4	98.3
5	98.4
6	98.5
RSD	**1.06 %**
Acceptance Criteria	Pass/Fail
% RSD ≤ 2.0 %	PASS

Intermediate Precision:

Intermediate precision was assessed by two analysts performing extractions using the ePrep ONE application. Mean assay values were comparable (± 2.0%) with overlapping ranges and %RSD within acceptable limits (Table 5), confirming robust intermediate precision.

Table 5 Summary of Intermediate Precision using ePrep ONE.

	Result (% Cyanocobalamin)			
Analyst	Range %	Average %	% RSD	Pass/Fail
Analyst 1	96.0 – 98.9	98.1%	1.06%	PASS
Analyst 2	96.8 – 98.3	97.5%	0.62%	PASS

Robustness:

Robustness was evaluated by introducing deliberate variations to stirring parameters (speed and time) and sample mass, followed by UV analysis. These parameters were selected based on their potential impact on extraction efficiency; CFR Part 11 software ensures operator-level permissions prevent unauthorised changes, with all modifications recorded via audit trail.

Assay results remained consistent with overlapping ranges across all conditions, confirming the robustness of the ePrep ONE extraction application.

Table 6 Robustness evaluation of the automated ePrep ONE application.

Robustness Criteria: Variation in Vortex Speed, Time and Sample Mass			
Sample	Average %	% RSD	Pass/Fail
-10% Vortex Speed & time +10% Sample Mass	99.0	0.08	PASS
Control	98.1	1.06	PASS
+10% Vortex Speed & time -10% Sample Mass	98.8	0.33	PASS

Linearity:

Linearity was evaluated using three replicate five-point curves spanning 50–150% of target concentration. All R^2 values exceeded 0.999 (Table 7), confirming the ePrep ONE automated extraction method is linear across the tested range.

Table 7 Linearity using ePrep ONE.

Preparation	Result R^2
1	0.9999
2	0.9998
3	0.9999
Acceptance Criteria	Pass/Fail
% RSD ≤ 2.0 %	PASS

Equivalency of Automated ePrep ONE Extraction:

Cyanocobalamin (USP) extracted via ePrep ONE and analysed on the METTLER TOLEDO UV7 showed no significant difference from the Certificate of Analysis value, meeting acceptance criteria.

Table 8 Comparison of Cyanocobalamin Content using ePrep ONE to COA

	COA	ePrep ONE			
Sample	Result (% w/w)	Result (% w/w)	% RSD	Specification	Pass/Fail
Cyanocobalamin	99.2	98.1	0.80 %	96.0 to 102.0 %	PASS

The results of the automated ePrep ONE extraction and COA are within 2.0%, as such the automated extraction is considered equivalent.

Considerations

This application is validated for cyanocobalamin content and identification by UV-Visible Spectroscopy per USP and BP monographs. The ePrep ONE – METTLER TOLEDO workflow provides a suitable automated extraction and UV-Vis approach for this determination.

The 21 CFR Part 11-compliant software enforces controlled user access, ensuring only authorised personnel can modify critical parameters. Robustness testing (varying vortex speed and time) confirmed no impact on assay content, demonstrating resilience to operational variations.

Without version-controlled applications or access restrictions, unauthorised modifications to extraction parameters risk non-conformant results. The ePrep ONE – METTLER TOLEDO solution mitigates this through enforced permissions, complete audit trails, standardised automated preparation, and unambiguous UV7 analysis, eliminating variability from analyst experience or manual technique.

Conclusion and Benefits

Automated B12 extraction using the ePrep ONE delivers targeted advantages for Cyanocobalamin quality control:

Financial & Efficiency: ePrep ONE automates stock preparation and dilution, eliminating the large sample masses, excess solvent, and analyst time required by manual volumetric methods, reducing cost per analysis and freeing capacity for higher-value work.

Regulatory Compliance & Data Integrity: As an expensive API vulnerable to counterfeiting and supply chain fraud, Vitamin B12 demands traceable site-level analysis. ePrep ONE's 21 CFR Part 11-compliant software ensures validated, audit-trailed extraction for every batch.

Environmental Sustainability: Automated workflows optimise reagent use and minimise solvent consumption and waste, reducing the ecological footprint of routine cyanocobalamin testing.

Analyst Safety & Wellbeing: Automation of repetitive volumetric steps reduces solvent exposure and repetitive stress risk, enabling analysts to focus on scientific interpretation.

The ePrep ONE elevates efficiency, compliance, sustainability, and safety of Vitamin B12 quality control at manufacturing scale.

The comparison of the manual extraction to the ePrep ONE automated workflow, demonstrating the potential benefits (Table 9)

Table 9 Comparison of preparation of 2 Samples Replicates by Manual to Automated ePrep ONE Analysis

Comparison Parameter	Manual Extraction	ePrep ONE extraction	% Reduction
Solvent Usage	1400 mL	80 mL	94 %
Duration	1.25 Hours	0.75 Hours	60 %
Hands on Time	1.25 Hours	20 Minutes	75 %

Ordering Information

Part numbers of the ePrep, accessories and spares used in this workflow:

Description	Part Number
ePrep ONE Sample Preparation Workstation Kit	01-01000
ePrep Vortex Mixer, 2P Version	01-04100
2 x Reagent Jar Adapter Plate/ Rack supplied with 5 X50 mL Jars	01-03085
Adapter Plate for 2x4 Microtiter Format, 20, 30, 40mL	01-03081
2 x 8 x 20-40 mL tube rack	01-03092
10mL ePrep Syringe	01-09026
1mL ePrep Syringe	01-09020

ePrep Pty Ltd
3 Kingston Town Close
Oakleigh, VIC, 3166
AUSTRALIA
(e) info@eprep.com.au
(w) www.eprep-analytical.com

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