

ePrep ONE® | *Application Note 2025* Automated Sample Extraction of Glycerin for Assay by Precision Titration

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Application Benefits

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Keywords

- Glycerin Assay
- USP Monograph
- BP Monograph
- EP Monograph
- 21 CFR Part 11
- Sustainability
- ePrep ONE
- Mettler Toledo T5

- **Regulatory Compliance:** The ePrep ONE and Mettler Toledo LabX T5 Autotitrator ensure Glycerin sample extraction and analysis meets USP, EP and BP monograph standards, minimizing regulatory risks and boosting research credibility.
- **Operational Efficiency:** By reducing manual tasks, ePrep ONE in partnership with the LabX T5 Autotitrator automates the analysis workflow, enabling scientists to focus on innovation and complete projects faster.
- **Sustainability:** ePrep ONE together with the Mettler T5 Autotitrator reduces resource use and waste, supporting green chemistry and sustainable lab operations without sacrificing performance.

Summary

This document describes the transition of glycerin sample extraction from traditional manual extraction and titration to an end-to-end automated process using the ePrep ONE Sample Extraction Workstation and Mettler Toledo LabX T5 Autotitrator. The aim is to streamline the quantification of glycerin analysis by precision titration, ensuring compliance to European Pharmacopoeia (EU), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) Monographs.

Key Objectives:

- Assess the compatibility of BP (Glycerin) and USP (Glycerin) monographs for glycerin assay by automation.
- Validate the automated workflow against manual extraction methods.
- Demonstrate equivalence in precision and accuracy to meet USP and BP compendial requirements.
- Enhance efficiency by reducing time, solvent usage, and labour.

The traditional manual assay of glycerin by titration described in pharmacopeia monograph is a labor intensive analysis. The reagents used are light sensitive and must be prepared on the day of analysis. The sample extraction has multiple reagent addition steps that must be precisely timed for satisfactory completion. Finally titration must be completed immediately due to the solution stability.

A key challenge in developing a validated automated extraction is balancing the need to minimize hands-on analyst time with reducing environmental impact. The process must ensure accurate and consistent results, significantly reduce solvent use and waste, and meet regulatory standards. There are a number of instruments available that can be used to automated sample extraction for chromatographic analysis, however, when extracting samples for precision titration finding a solution is more challenging. Most automated sample extraction systems lack the flexibility needed to prepare and deliver an adequate sample volume directly to the titration beaker, or focus on a single workflow stream making them less practical and cost effective solution in a laboratory performing multiple analysis methods.

The automated solution discussed in this paper leverages the connectivity of the Mettler Toledo LabX instrument range with the flexibility of the ePrep ONE to deliver an end-to-end automated workflow. The samples for analysis are scanned using a barcode reader connected to Lab X. The samples are accurately weighed, with the masses recorded using a Mettler Toledo Analytical Balance. The samples are placed onto the ePrep ONE, where the barcoded titration beaker is fitted into the ePrep ONE autotitrator® sample rack.

The ePrep ONE completes the preparation of the reagents required for extraction, times the addition of reagents to perfection, seamlessly extracting the sample without analyst intervention. The titration beaker is scanned and placed onto the T5 Autotitrator for precision titration. On completion of the titration, the Lab X software completes the calculation of results, without any intervention by the Analyst. The ePrep ONE application has been specifically designed to complete the blank and sample replicates at precisely the exact moment they are needed for titration, minimizing the risk of failure due to solution stability.

Several critical parameters, such as precision, linearity, and limit of detection, were evaluated. The results showed that the automated method performed as well or better than manual methods. Additionally, the automated process reduces the use of hazardous



chemicals, improves safety by minimizing human exposure to potentially harmful solvents, and lowers operational costs through automation.

The document concludes that switching to an automated extraction using ePrep ONE and Autotitration analysis with Mettler Toledo LabX offers significant benefits, including time, solvent, and cost savings, improved precision and accuracy, and reduced environmental impact, all while providing a 21 CFR Part 11 compliant validated pharmacopeia-equivalent workflow.

ePrep ONE & Mettler Toledo Automated Precision Titration

The ePrep ONE is a syringe-based analytical system designed for precise handling of solvents and sample matrices. With syringes ranging from 10 μ L to 10 mL, it ensures accurate volumes as low as 1 μ L. Its CFR Part 11 validated software simplifies optimization of parameters like volume and flow rate, while maintaining GMP compliance with a full audit trail.

Using positive displacement, the syringes handle non-aqueous liquids effectively. Stainless-steel needles prevent solvent contamination and reduce single-use plastic waste, supporting sustainability goals. Manual preparation often risks OOS or OOT results, leading to costly delays. Automation with the ePrep ONE minimizes these risks, allowing skilled staff to focus on critical tasks.

The system ensures consistent results regardless of expertise of the available operator, thanks to its programmed workflows. Its intuitive software enables quick workflow creation with drag-and-drop tools, predefined coordinates, and reusable workflows, reducing setup time and enhancing lab efficiency.

The ePrep ONE revolutionizes sample extraction automation for precision titration, offering unmatched flexibility and accuracy. This first-of-its-kind system ensures precise preparation of titration-ready samples, whether as a single, time-sensitive extraction or a complete sample set optimized for seamless auto-sampler integration. By delivering consistent, high-quality results, the ePrep ONE empowers laboratories to achieve unparalleled efficiency and reliability in titration workflows.



Figure 1: Automated Extraction and Assay of Glycerin using ePrep ONE & Mettler Toledo



Mettler-Toledo LabX™ consolidates all Mettler Toledo instruments into one interface, by leveraging the connectivity of the Analytical Balance and the Autotitrator together. Combining this with CFR Part-11 ePrep ONE software there is complete digital traceability of the sample handling from the first measurement to the final result. The combined solution offers unmatched regulatory compliance.

Materials and Instrumentation

All samples and standards were weighed as shown in Table 1, using an analytical balance (Mettler Toledo). They were then prepared using the fully automated sample extraction instrument, ePrep ONE. The equipment needed for performing titration extraction on ePrep ONE is listed in Table 2.

Table 1 Reagents and Solvents required for Assay of Glycerin

Name	Grade	Brand	Mass
Sodium Periodate	AR	Chem Supply	1.0700 ± 0.1070 g
0.1 M Sulphuric Acid	AR	Sigma Aldrich	-
Ethanediol (polyethylene glycol)	AR	Chem Supply	10.0000 ± 0.1000 g
0.1M Sodium Hydroxide <i>Standardized</i>	AR	Sigma Aldrich Titripur	-
Purified Water	RO	-	-
Sample for Analysis	BP	-	0.0300 ± 0.0030 g

Table 2 Equipment required for required for Assay of Glycerin using ePrep ONE and Mettler Toledo T5

Glassware	Syringes/Tools	Decks
50 mL Amber Glass Reagent Jars	10 mL Standard Syringe	Reagent Jar Rack
20 mL Amber Glass Vials	1 mL Standard Syringe	Vortex Mixer Rack
100 mL Autotitration Beaker Red	5 mL Probe Dispenser	ePrep ONE Autotitrator Rack
1L Schott Bottle	DGi115-SC Electrode	

Following extraction of all Samples and Standards, analysis was performed using precision titration, Mettler Toledo T5 Autotitrator using the conditions specified in table 3.

Table 3 T5 Autotitrator Conditions

Titration Conditions	
Autotitrator	Mettler Toledo®, T5
Autotitrator Probe	DGi115-SC Electrode
Temperature	25°C
Titrant	0.1M Sodium Hydroxide (NaOH)
Burette Volume	20 mL



Titration Program

Step	Parameter	
001	Type	General Titration
	Compatibility	T5/T7/T9
	Titration Type	pH
	Pre-stir Duration	60 sec
	Relevant EQP	1
	Control	Normal
	Stir	35%
	Pre-dispense	0 mL
	Calculation	Content %
002	Sample Type	Sample
	Number of IDs	1
	Entry Type	Weight
	Lower Limit	0.0 g
	Upper Limit	5.0 g
	Density	1.0 g/mL
	Number of Sample Factors	0
	Correction Factor	1.0
	Temperature	25°C
	Entry	Arbitrary
	Titration Reader	None
	003	Titration Stand
004	Stir	
	Stir Speed	35%
	Stir Duration	60 sec
005	Titration (EQP)	
	Titrant	
	Titrant	NaOH
	Concentration	0.1 mol/L
	Sensor	
	Type	pH
	Sensor	DGi115-SC
	Unit	pH
	Temperature Acquisition	
	Temperature Acquisition	Not Applicable
	Stir	
	Speed	35%
	Pre-dispense	
	Mode	None
	Wait Time	0 s
	Control	
	Control	User
	Mode	Dynamic
	Show Parameters	Not Applicable
	Evaluation and Recognition	
	Procedure	Standard
Threshold	3.0 pH/mL	



Titration Program

Step	Parameter	
	Tendency	Positive
	Ranges	0
	Add EQP Criteria	None
	Termination	
	At VMax	20mL
	At Potential	Yes
	Potential	9
	At Slope	No
	After Number of EQPs	No
	Combined Termination Criteria	No
	Calculation of R1 Consumption	
	Result	Consumption
	Result Unit	mL
006	Formula	R1VEQ
	Constant	C=1
	M	M [Sodium Chloride]
	Z	Z [Sodium Chloride]
006	Decimal Places	5
	Result Limits	Not Applicable
	Record Statistics	Yes
	Send to Buffer	Not Applicable
	Write to Smart Tag	None
007	Calculation R2: Content	
	Result	Content
	Result Unit	%
	Formula	R1=Q-B[Glycerin]; mmol R2=(Q-B[Glycerin])*C/m C=1/z ; mol/L B[Glycerin]: Blank Value
	Decimal Places	5
	Result Limits	Not Applicable
	Record Statistics	Yes
	Extra Statistical Functions	Not Applicable
	Send to Buffer	Not Applicable
	Write to Smart Tag	None



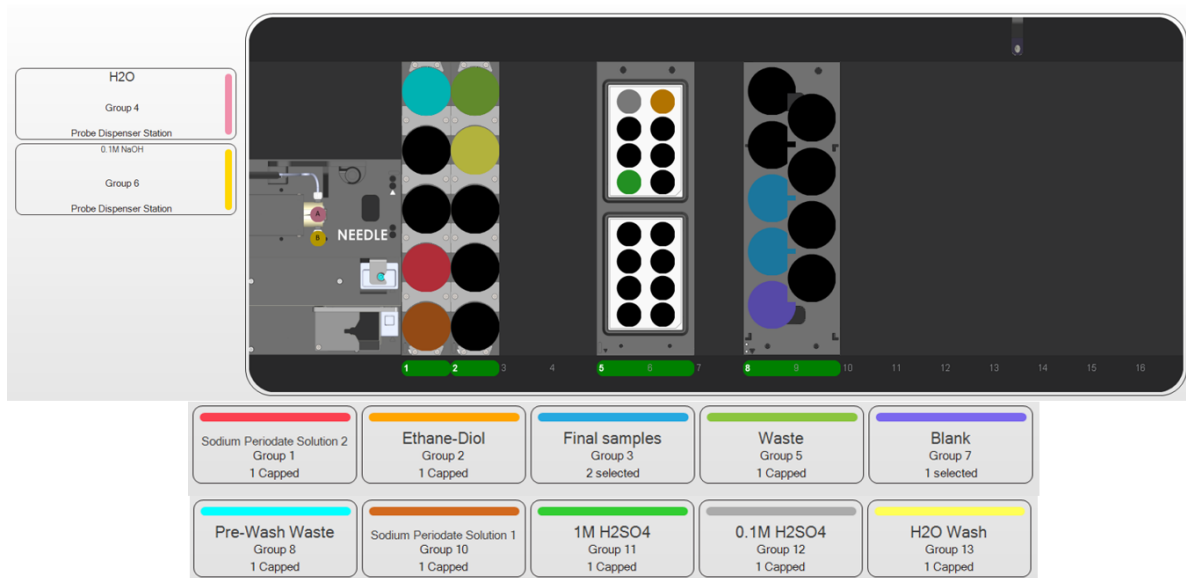


Figure 2 Screen capture of the ePrep ONE deck for Assay of Glycerin (2 samples + 1 blank)

1A	Add 2.5 mL of 1M H2SO4 to Solution 1 10000 uL Syringe - AP98Y	Diluent Volume 2500 µL	1M H2SO4 Group 11	Sodium Periodate Solution 1 Group 10
1B	Make Up to 50 mL with H2O 5000 uL Dispenser	Make Up To 50000 µL	H2O Acc - Group 4	Sodium Periodate Solution 1 Group 10
1C	Mix Sodium Periodate Solution 1			
2A	Aliquot 40 mL to Solution 2 10000 uL Syringe - AP98Y	Sample Volume 40000 µL	Sodium Periodate Solution 1 Group 10	Sodium Periodate Solution 2 Group 1
2B	Add 2.0 mL of 0.1M H2SO4 to Solution 2 10000 uL Syringe - AP98Y	Reag Volume 2000 µL	0.1M H2SO4 Group 12	Sodium Periodate Solution 2 Group 1
2C	Syringe Mix Solution 2 10000 uL Syringe - AP98Y	Mix Volume 2000 µL		Sodium Periodate Solution 2 Group 1
3A	Add 10.0 mL of H2O to Ethandiol 5000 uL Dispenser	Diluent Volume 10000 µL	H2O Acc - Group 4	Ethane-Diol Group 2
3B	Vortex Mix	Mix Time / RPM 300 Sec / 500		Ethane-Diol Group 2
4	Final Samples Make Up To Volume with H2O 5000 uL Dispenser	Make Up To 18000 µL	H2O Acc - Group 4	Final samples Group 3
5	Tool Wash 10 mL Syringe with Solution 2 10000 uL Syringe - AP98Y	Diluent Volume 1 µL	Sodium Periodate Solution 2 Group 1	Completion Vial Group 7
6A	Add 10 mL of Solution 2 to Final Samples 10000 uL Syringe - AP98Y	Reag Volume 10000 µL	Sodium Periodate Solution 2 Group 1	Final samples Group 3
6B	Pause for 5 Minutes	Pause Time 5 min		
7	Pause for 0.67 Minutes	Pause Time 0.67 min		
8A	Add 2 mL of Ethandiol to Final Samples 10000 uL Syringe - AP98Y	Reag Volume 2000 µL	Ethane-Diol Group 2	Final samples Group 3
8B	Pause for 5.5 Minutes	Pause Time 5.5 min		
9	Pause for Blank Sample	Pause Time 3.5 min		
10	Add 5 mL of NaOH to Blank 5000 uL Dispenser		0.1M NaOH Acc - Group 6	Final samples Group 3
11	Blank Sample is now ready to be analysed. 5000 uL Dispenser	Diluent Volume 1 µL	0.1M NaOH Acc - Group 6	Completion Vial Group 7
12	Pause for Sample A	Pause Time 6 min		
13	Add 7 mL of NaOH to Sample A 5000 uL Dispenser		0.1M NaOH Acc - Group 6	Final samples Group 3
14	Sample A is now ready to be analysed 5000 uL Dispenser	Diluent Volume 1 µL	0.1M NaOH Acc - Group 6	Completion Vial Group 7
15	Pause for Sample B	Pause Time 6 min		
16	Add 7 mL of NaOH to Sample B 5000 uL Dispenser		0.1M NaOH Acc - Group 6	Final samples Group 3



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

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 an in-house validated Ego Pharmaceuticals method based on the BP monograph. The BP monograph conditions are identical to the conditions specified in the EP and USP monographs. The monograph method involves a dissolution of glycerin in water, addition of sodium periodate followed by a 30 minute reaction step. A subsequent 20 minute reaction step is completed following the addition of ethanediol to create the final sample solution. The resultant sample must be titrated immediately following completion of the second reaction step.

Sample Extraction

In the automated ePrep ONE application, the sample identification is scanned using Lab X. 0.0300 g of the sample for analysis is weighed into a 100 mL titration beaker and placed by the Analyst onto the ePrep ONE instrument group 3, deck position 8, Figure 2. A 100 mL titration beaker for the blank solution is also placed onto the deck, group 7, deck position 8, Figure 2.

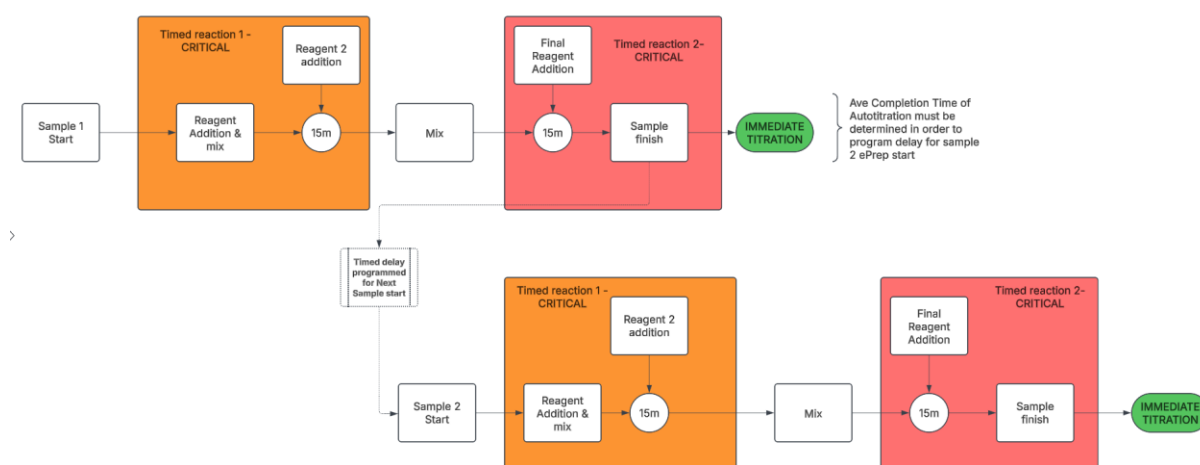


Figure 4 Simplified Process Flow of ePrep ONE Sample Extraction Application Design

The automated ePrep ONE extraction application uses the 10 mL syringe to add 2.5 mL of 1M sulphuric acid to the 50 mL reagent jar containing 1.070 g of sodium periodate, sample jar, group 1, deck position 1, Figure 2. The probe dispenser, group 4, line A, figure 2, is then



used to accurately make the volume to 50 mL with diluent (water) and mixed to create sodium periodate solution 1. A 40 mL aliquot is taken from the initial preparation and mixed with an additional 2 mL of 0.1 M sulphuric acid, followed by syringe mixing, to create sodium periodate solution 2, group 1, deck position 10, Figure 2.

Following the preparation of the sodium periodate solution the ePrep ONE application completes the preparation of the ethanediol reagent. The probe dispenser group 4, line A, figure 2, is used to add 10 mL of diluent (water) to the ethanediol solution group 2, deck position 5, Figure 2. The solution is then vortex mixed by the ePrep ONE for 5 minutes at 500 RPM.

The preparation of the reagents by the ePrep ONE process ensures that the reagent solutions required for the analysis are always freshly prepared, each time the application is run without adding additional preparation tasks to the analyst's schedule. It also minimises the use of chemicals and waste produced by the laboratory.

The ePrep ONE uses the probe dispenser group 4, line A, figure 2, to add 18 mL of diluent (water) to the sample titration beakers group 3, deck position 8, Figure 2. The ePrep ONE 10 mL syringe is used to deliver sodium periodate solution into the blank titration beaker group 7, deck position 8, Figure 2 and the sample titration beakers group 3, deck position 8, Figure 2. The ePrep ONE then pauses to begin the first timed reaction.

After completion of the initial pause, 2 mL of ethanediol is added to the sample titration beakers group 3, deck position 8, Figure 2. The ePrep ONE then completes an additional pause step before adding 5 mL of 0.1 M sodium hydroxide from probe dispenser line B, group 6, figure 2 to the sample titration beakers, group 3, deck position 8, Figure 2. Following addition of 0.1 M sodium hydroxide to the samples, the probe dispenser adds 0.1 M sodium hydroxide to the blank titration beaker, group 7, deck position 8, Figure 2. The ePrep ONE alerts the Analyst that the blank sample is now ready for presentation to the autotitrator.

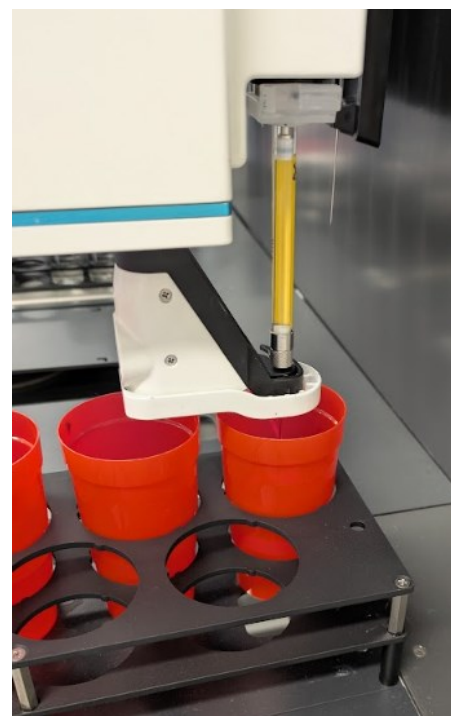


Figure 5 ePrep ONE Mettler Toledo Titration Rack

Whilst the titration of the blank is completed, the ePrep ONE continues preparation of the samples, an additional pause step is completed before 7 mL of 0.1 M sodium hydroxide from probe dispenser line B, group 6, figure 2 is added to Sample A, group 3, deck position 8, Figure 2. The ePrep ONE alerts the Analyst that Sample A is now ready for presentation to the autotitrator.

Whilst the titration of Sample A is completed, the ePrep ONE continues preparation of Sample B, and when complete it is now ready for presentation to the autotitrator.



The solution concentrations are maintained to follow the BP Pharmacopeia and Mettler-Toledo auto-titration guidelines, ensuring the titre is within the burette volume range and optimising solvent use by the ePrep ONE. The development team optimized the pause steps within the ePrep ONE application to strictly adhere to the reaction times specified within the monograph whilst also allowing the Analyst time to complete the titration.

ePrep ONE System Parameters

The following Parameters will be discussed in detail, however a summary of the selected parameters are in Table 5.

Table 5 Summary Table for Parameters of ePrep ONE Sample extraction

Parameter	Chosen Option
Vial Choice	50 mL Amber Glass Vials
	20 mL Amber Glass Vials
	100 mL Titration Beaker Red
Probe Dispenser	5 mL Probe Dispenser
Needle Choice	10 mL ePrep ONE Syringe
Aspiration Speed ($\mu\text{L}/\text{Sec}$)	175
Aspirate Pause (Sec)	1
Dispense Flow Rate ($\mu\text{L}/\text{Sec}$)	500
Dispense Pause (Sec)	1
Waste	ePrep ONE wash station
Mixing Type	Vortex Mixing
	Syringe Mixing

Vial and Needle Choice

In this study, 50 mL and 20 mL Amber Glass Vials were selected to complete the extraction, the amber glass vials ensure the reagents are protected from light consistent with the monograph preparation conditions and to support efficient extraction. A standard needle size syringe is used in combination with the 20 mL amber glass vials, for accurate sample collection. RFID identification of the required syringe, combined with platform setup verification by the CRF Part 11 validated instrument software, ensures that errors from incorrect apparatus are avoided. This process enhances accuracy and reliability before commencing operations. The 100 mL Autotitration beaker (red) from Mettler-Toledo is used to ensure that the sample extraction is completed as efficiently as possible with no sample transfer required.

Aspiration and Dispense

Speed, Pause Time and Dispense flow rate: The speed and duration of aspiration and dispense ensure optimal performance during the sample extraction process. For the aspiration speed, 175 $\mu\text{L}/\text{sec}$ was selected, it was determined that using this aspiration speed setting did not introduce sample variability. The dispense



parameters of the aspirated sample, are less critical than those used during aspiration. The dispense speed was set to 750 $\mu\text{L}/\text{Sec}$ with a pause time of three seconds. The pause time in addition to aiding the sample draw also allows for any droplets formed on the syringe needle to be dispensed. By configuring the needle depth to Auto Low, we ensure that droplets do not accumulate on the syringe, as the needle is immersed in the pre-delivered solvent, this approach helps maintain the integrity of the sample transfer process.

Pause Settings

The pause settings configured by the development team are critical to the completion of the reaction steps defined within the pharmacopeia method. Careful consideration was given during development to ensure that the time taken by the instrument to complete dispensing steps, in addition to the programmed pause settings in the application steps matches the reaction times following reagent addition described in the monograph. In addition to ensuring the timed reaction steps were optimal the development team gave consideration to the time required for the analyst to complete the titration.

Waste

The ePrep ONE wash station is configured to wash the system during execution of the sample extraction application. The wash settings are designed to remove residue from the ePrep ONE probe dispenser and syringes prior to preparation of titration samples, and following the tool use to ensure the instrument is in perfect condition for the next use.

Results and Discussion

The results from the application validation of the automated extraction of glycerin are outlined below. The specification applied to the analysis is that of the British Pharmacopeia (BP) 98.0 to 101.0% based on the anhydrous basis.

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

Precision of Sample extraction: A set of six samples will be prepared using the ePrep ONE workflow, with results analysed for relative standard deviation (RSD), the limit for the RSD of 6 sample preparations is $\leq 2.0\%$.

Intermediate Precision: The RSD between first and second analyst is $\leq 2.0\%$.

Robustness: The RSD between initial precision and various robustness parameters is $\leq 2.0\%$.

Accuracy: The results obtained from samples prepared using the ePrep ONE automated extraction is $\leq 2.0\%$ of results obtained from manual extraction.

Equivalency of Automated ePrep ONE to Manual Extraction: Samples from a single batch of Glycerin (BP) were extracted using manual extraction and using the automated ePrep ONE application. The analysis by titration reveals no significant difference between manual and



automated extraction. Additionally, a comparison of the automated ePrep ONE extracted sample and the result given on the Certificate of Analysis (COA) reveals no significant difference, thereby meeting the acceptance criteria.

Table 6 Comparison of Glycerin Content using manual extraction and ePrep ONE

Sample	COA Result (% w/w)	Manual Extraction Result (% w/w)	ePrep ONE Result (% w/w)	% RSD	Specification	Pass/Fail
Glycerin	100.3	99.5	99.8	0.40 %	98.0 to 101.0 %	PASS

The results obtained using the automated ePrep ONE extraction were compared to the content result obtained from manual extraction of the same batch as well as to the results obtained from the certificate of analysis. All results for both the ePrep ONE and manual extraction methods were within specification. The results of the automated ePrep ONE extraction are within 2.0% of the results when extracted manually, as such the automated extraction is considered equivalent to the manual extraction method.

Precision (Repeatability): The precision of the automated sample extraction using ePrep ONE was evaluated by performing six replicate extractions from the same sample under the proposed application. The analysis of the extracted samples yielded consistent content results, with a %RSD of 0.76% across the six replicates, as shown in Table 7.

All results were within the predefined specification limits, demonstrating that the automated ePrep ONE extraction method is precise.

Table 7 Precision using ePrep ONE.

Preparation	Result (% Glycerin)
1	99.8
2	100.5
3	101.7
4	99.8
5	100.3
6	99.6
RSD	0.76 %
Acceptance Criteria	Pass/Fail
% RSD ≤ 2.0 %	PASS

Intermediate Precision: Intermediate precision was assessed by two independent analysts performing sample extraction using the proposed automated ePrep ONE application. The mean assay values obtained by each analyst (mean ± 2.0%) were comparable, and the results showed overlapping ranges. The %RSD between analysts was within acceptable limits, indicating good intermediate precision. These results support the method's validation with respect to intermediate precision. A summary of the data is provided in Table 8.



Table 8 Summary of Intermediate Precision using ePrep ONE.

Analyst	Result (% Glycerin)		% RSD	Pass/Fail
	Range %	Average %		
Analyst 1	98.2 – 102.2	100.2%	0.49%	PASS
Analyst 2	98.5 – 102.6	100.6%	0.63%	PASS

Robustness: Robustness of the automated ePrep ONE sample extraction application was evaluated by introducing deliberate variations to critical method parameters, followed by titration of the resulting samples.

The modifications included (i) adjusting the aspiration settings used within the application, (ii) varying the time from completion of sample to completion of titration. These parameters were selected by the development team based on their potential impact on extraction efficiency. During development it was identified that the pause settings are critical to the completion of the extraction. As the ePrep ONE application is designed to be completed using CFR Part 11 software, the user role permissions may be configured to ensure that the pause settings of the application cannot be altered by the operator. Any changes made to the application are recorded by the audit trail. The time of completion of the sample is known and the presentation of the sample to the autotitrator is recorded by the LabX software, meaning that full sample traceability is maintained.

The development team determined that the time taken between the completion of the ePrep ONE application and the presentation of the sample to the autotitrator was at the discretion of the Analyst, therefore an experiment was conducted to determine the maximum amount of time the samples can be held prior to titration. This information may be useful for laboratories wishing to use autotitrators configured with an autosampler, however for the purposes of the study manual sample presentation was used. The results obtained under the modified conditions were compared to those from the original precision study.

The assay results for both the aspiration study and the sample stability following standing of up to 15 minutes remained consistent, with overlapping ranges observed across all conditions. No significant differences in sample content were detected, confirming the robustness of the ePrep ONE extraction application under the tested conditions.

Table 9 Robustness evaluation of the automated ePrep ONE application.

Robustness Criteria: Variation in Aspiration and Standing time			
Sample	Average %	% RSD	Pass/Fail
Aspiration Study			
-2% Aspiration Speed	99.8	1.06	PASS
Control	100.2	0.49	PASS
+2% Aspiration Speed	100.1	1.00	PASS
Solution Stability Study			
Control	100.2	0.49	PASS
5 minutes	100.4	0.92	PASS
10 minutes	99.9	1.95	PASS
15 minutes	99.7	0.72	PASS



Considerations

The application discussed in this white paper has been validated against the in-house method for determination of glycerin content by precision titration based on the BP monograph (*Glycerol*). In addition to the validation criteria, the performance of manual extraction vs the automated ePrep ONE application was assessed. The development team believe that the ePrep ONE - Mettler Toledo workflow would be a suitable automated extraction and precision titration approach for determination of Glycerin content.

Timing of the reactions steps for the extraction is critical to the assay of Glycerin. The ePrep ONE software precisely controls the addition of reagents throughout the application, it should be noted that the timing steps were meticulously modified during development of the application, any modifications made to the application as written would need to verify that the reaction timings were not altered as a result.

Variations in timing of the reagent addition were seen to have the potential to impact extraction efficiency and, consequently, analyte content during development. To mitigate this risk, the ePrep ONE employs CFR Part 11-compliant software with controlled user access and permission levels. This ensures that only authorized personnel can modify critical application parameters, thereby preventing both accidental and deliberate alterations that could compromise method performance. Furthermore, as part of the method validation, robustness testing was conducted by varying aspiration speed and standing time following completion. The results demonstrated no impact on the titration, confirming that the method is resilient to such operational variations.

Organizations that operate without robust, version-controlled applications or user access restrictions, such as those provided by CFR Part 11-compliant software, must consider the risks associated with unauthorized modifications to extraction parameters. As demonstrated during development testing, even minor, uncontrolled changes to critical extraction settings can lead to non-conformant results.

The ePrep ONE - Mettler Toledo solution described in this paper mitigates this risk through the integrated CFR Part 11-compliant software platforms, which enforce user permission levels and maintain complete audit trail. This ensures that all method changes are either restricted or traceable. Furthermore, the fully automated ePrep ONE application standardizes sample preparation, while the Mettler Toledo T5 autotitrator any ambiguity from titration end point determination, effectively eliminating variability introduced by differences in analyst experience or manual technique.

Benefits

Automation in sample extraction for precision titration heralds transformative financial, Greenlab environmental sustainability, and human advantages. In addition, the ePrep ONE 21 CRF Part 11 software ensures compliance, by providing a validated automated sample extraction that meets stringent quality and regulatory standards. The ePrep ONE automates the extraction process, reducing human intervention and minimizing errors, while maintaining precision and accuracy. The ePrep ONE not only enhances operational



efficiency but also ensures that laboratory practices align with regulatory requirements, thus safeguarding data integrity and reliability.

The ePrep ONE system can revolutionize laboratory practices by optimizing reagent use, minimizing waste, and reducing solvent consumption and waste generation, easily achieving sustainability goals and decreasing the ecological footprint. For Analysts, the ePrep ONE system liberates them from monotonous repetitive tasks, enhancing job satisfaction and enabling focus on more intricate scientific endeavours. It significantly boosts safety by minimizing exposure to hazardous substances and reducing lost time due to repetitive stress injuries.

In essence, automated sample extraction using ePrep ONE, elevates efficiency, sustainability, and workplace safety, profoundly benefiting both the laboratory environment and its dedicated professionals.

The comparison of the manual extraction to the ePrep ONE automated workflow, demonstrating the potential benefits are shown in Table 10

Table 10 Comparison of preparation of 2 Samples Replicates by Manual to Automated ePrep ONE Analysis

Comparison Parameter	Manual Extraction	ePrep ONE extraction	% Reduction
Solvent Usage	330 mL	140 mL	58 %
Duration	4 Hours	1.5 Hours	63 %
Hands on Time	4 Hours	20 Minutes	92 %

Supplementary Data

Table 11 Settings for 5000 µL Dispenser

Settings for 5000 µL Dispenser	
Aspirate Flow Rate (µL/sec)	2500
Aspirate Pause (Sec)	2
Dispense Flow Rate (µL/sec)	750
Dispense Pause (Sec)	3
Purge Settings	
Purge to	Waste
Purge Volume (µL)	2500
Aspirate Flow Rate (µL/sec)	2500
Aspirate Pause (Sec)	2
Dispense Flow Rate (µL/sec)	750
Dispense Pause (Sec)	3
Purge Cycles	3



Table 12 Settings for 10 mL Syringe

Settings for 10 mL Syringe	
Aspirate Flow Rate ($\mu\text{L}/\text{sec}$)	175
Aspirate Pause (Sec)	2
Dispense Flow Rate ($\mu\text{L}/\text{sec}$)	500
Dispense Pause (Sec)	2
Tool Wash Settings	
Tool Wash Volume (μL)	800
Tool Wash Aspirate Flow Rate ($\mu\text{L}/\text{Sec}$)	175
Wash Aspirate Pause (Sec)	1
Tool Wash Dispense Flow Rate ($\mu\text{L}/\text{Sec}$)	500
Wash Dispense Pause (Sec)	1
Tool Wash Cycles	2
Wash Piercer After Tool Wash	NO
Prime Settings	
Prime Method	To Tube
Prime Volume (μL)	1000
Prime Aspirate Flow Rate ($\mu\text{L}/\text{Sec}$)	175
Prime Aspirate Pause (Sec)	2
Prime Dispense Flow Rate ($\mu\text{L}/\text{Sec}$)	500
Prime Dispense Pause (Sec)	0
Prime Cycles	2
Prime Cycles When Refilling	1

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