Application

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ePrep ONE® | Application Note 2026
Automated Sample Preparation Assay of Hydrocortisone Solution

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Keywords
- USP Monograph
- CFR Part 11
- Sustainability
- ePrep ONE
- Shimadzu Prominence HPLC

Application Benefits

- Workflow provides automated sample preparation of Assay of Hydrocortisone Solution
- Reduces manual handling, improves efficiency, reduces environmental impact and provides significant cost savings compared to manual extraction.
- Meets stringent regulatory and data integrity requirements of modern laboratories

Application Purpose

This application, developed collaboratively by Ego Pharmaceuticals and ePrep Australia, utilises the ePrep ONE Sample Preparation Platform to automate the extraction of Hydrocortisone Solution for assay by High Performance Liquid Chromatography (HPLC).

Key Objectives:

- Develop an automated sample preparation of Hydrocortisone Solution for analysis by HPLC.
- Validate the automated ePrep ONE sample preparation against established manual methods using precision, linearity, and robustness criteria.
- Introduce full 21 CFR Part 11-compliant traceability to the sample extraction process.
- Improve laboratory efficiency by reducing extraction time, hydrocarbon solvent consumption, and manual labour.

Summary

The ePrep ONE demonstrated equivalent or superior performance to manual sample preparation across all validated parameters. Transitioning to automated sample preparation delivers reduced solvent use and waste generation, enhanced data integrity through end-to-end traceability, lower per-sample cost, and improved consistency between operators, while maintaining a fully validated, 21 CFR Part 11-compliant workflow.

Introduction

Hydrocortisone is an established corticosteroid that mimics cortisol, providing temporary relief of mild inflammatory skin conditions including eczema, dermatitis, contact allergies, insect bites, psoriasis, and sunburn.

As a regulated pharmaceutical, Hydrocortisone places significant analytical scrutiny on laboratories providing finished-product testing. Sample extraction remains a common gap in end-to-end traceability. It is also one of the most frequent sources of laboratory rework: manual extraction errors are difficult to attribute to a verifiable root cause, leading to costly repeat analyses and, in severe cases, regulatory citations such as FDA 483 observations.

Sample Preparation

In the automated ePrep ONE application, the standard, reference matrix and samples for analysis are weighed and quantitatively transferred into 20 mL glass vials and placed by the Analyst onto the ePrep ONE deck, Figure 2.

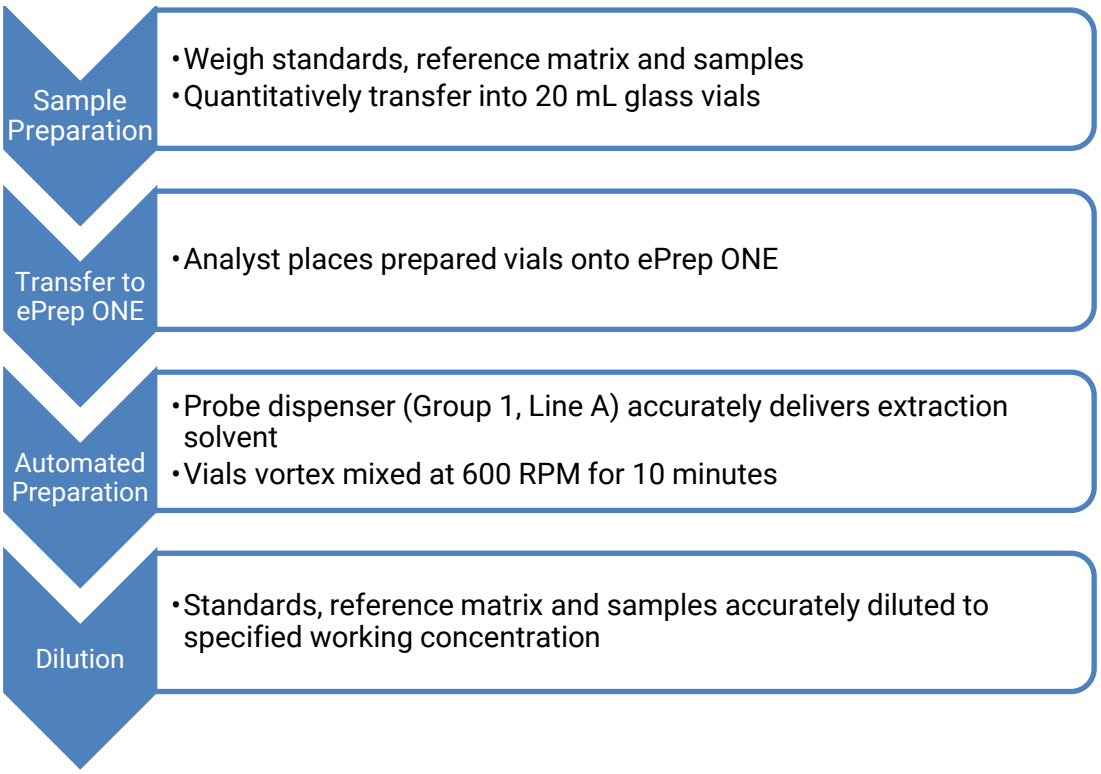

Figure 1 Automated sample extraction process flow for Hydrocortisone Solution assay using the ePrep ONE

The ePrep ONE application uses the probe dispenser, Figure 3, group 1, first line, to accurately dispense 20 mL of diluent (methanol) into each vial. The delivery speed of the diluent is precisely controlled to minimise foaming of the sample. The vials are vortex mixed at 600 RPM for 10 minutes.

Following the initial vortex mix, the standards, reference matrix and samples are accurately diluted to the working concentration specified in the manual extraction method, to maintain equivalency with the methodology described in the pharmacopeia.

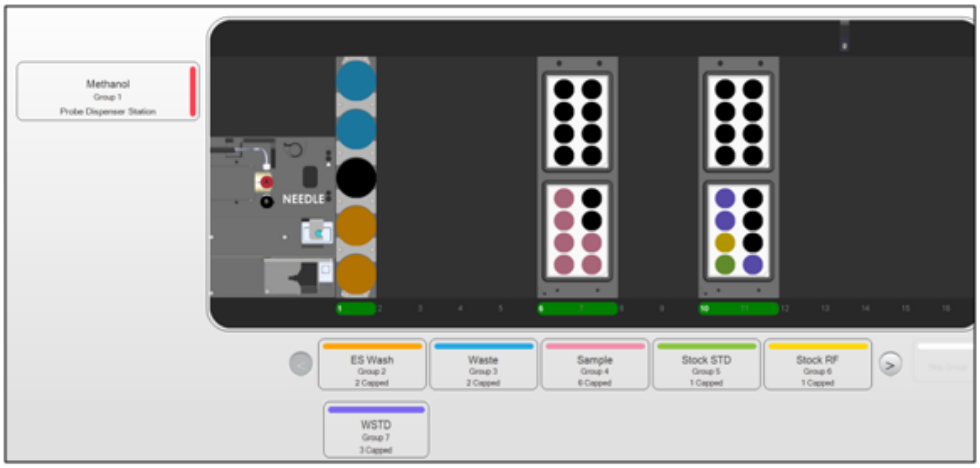

Figure 2 ePrep ONE deck configuration

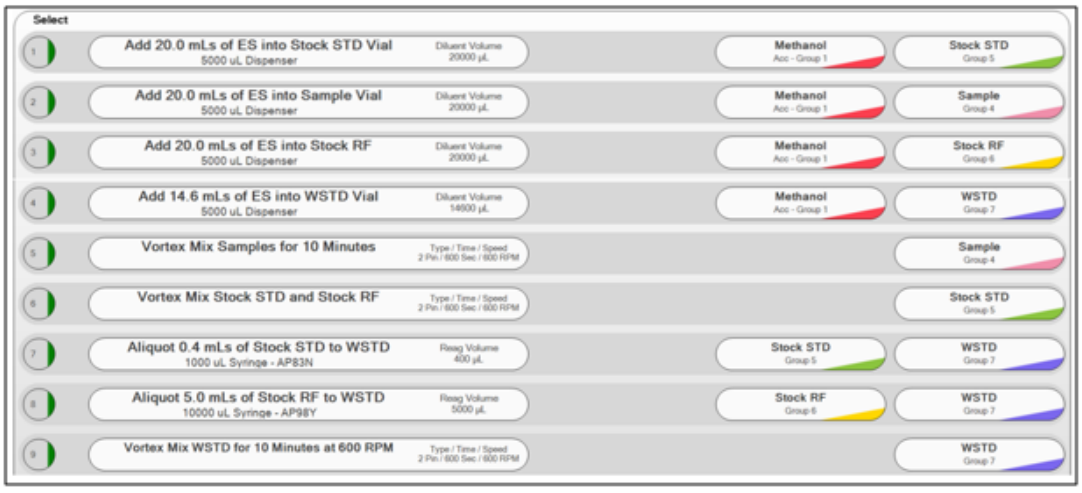

Figure 3 ePrep ONE application

Results and Discussion

The automated ePrep ONE extraction application was validated against the manual extraction method for Hydrocortisone using Shimadzu Prominence HPLC. The in-house method is based on the United States Pharmacopeia (USP) monograph for Hydrocortisone Lotion.

Accuracy (Recovery): The accuracy was assessed using the following criteria, the RSD between P1 to P6 is within 2.0 %. The RSD between the injections of each sample solution is within 2.0 %. The RSD between extreme results (lowest and highest result) is within 2.0 %.

Table 1 Accuracy (Recovery) Results

Sample	Result (% Hydrocortisone)		
T 1	1.0036		
T 2	0.9996		
DM 1	1.0031		
DM 2	1.0070		
IM 1	1.0048		
IM 2	1.0093	Outcome	
Average	1.0046%	% RSD: 0.33	PASS
	Extreme weight % RSD	% RSD: 0.27	PASS
	Extreme result % RSD	% RSD: 0.70	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.

Table 2 Summary of Intermediate Precision using ePrep ONE.

	Result (% Hydrocortisone)			
Analyst	Range %	Average %	% RSD	Pass/Fail
Analyst 1	0.99 – 1.03	1.0074%	0.00%	PASS
Analyst 2	0.98 – 1.02	1.0016%	0.0028%	PASS

Equivalency of Automated ePrep ONE to Manual Extraction:

Two Duplicate samples were extracted. The first duplicate sample was extracted using manual extraction, the second duplicate, using the automated ePrep ONE workflow. The analysis by HPLC reveals no significant difference between manual and automated extraction. The automated extraction is considered equivalent to the manual extraction method.

Table 3 Comparison of Hydrocortisone content using manual extraction and ePrep ONE

	Manual Extraction	ePrep ONE		
Analyte	Result (%)	Result (%)	% RSD	Pass/Fail
Hydrocortisone	1.0174	1.0078	0.68 %	PASS

Benefits

Automation in sample extraction delivers transformative financial, environmental, and human benefits. The ePrep ONE system, with 21 CFR Part 11-compliant software, ensures validated, precise, and accurate extraction while reducing human intervention and errors. It enhances operational efficiency, safeguards data integrity, and aligns laboratory practices with stringent regulatory standards, ensuring reliability and compliance.

The ePrep ONE revolutionizes laboratories by optimizing reagent use, minimizing waste, and reducing solvent consumption, supporting sustainability and lowering ecological impact. For analysts, it eliminates repetitive tasks, boosts job satisfaction, and enhances safety by reducing exposure to hazards and injury risks. This system drives efficiency, sustainability, and safety, creating a positive impact on both the environment and laboratory professionals.

The comparison of the manual extraction to the ePrep ONE automated workflow, demonstrating the potential benefits are shown in Table 4

Table 4 Comparison of preparation of 2 Samples Replicates by Manual to Automated ePrep ONE Analysis

Comparison Parameter	Manual Extraction	ePrep ONE extraction	% Reduction
Solvent Usage	350 mL	80 mL	77 %
Fume Hood Use	2 Hours	0 Minutes	100 %
Duration	2 Hours	50 Minutes	58 %
Hands on Time	2 Hours	10 Minutes	92 %

Conclusion

The successful transfer from manual to automated extraction using the ePrep ONE system for hydrocortisone analysis by HPLC highlights significant gains in precision, reproducibility, and operational efficiency. Operating within a CFR Part 11–compliant environment ensures robust control of user access and method parameters, minimising deviations from validated procedures and strengthening data integrity.

Validation confirms that automated sample preparation delivers consistent, accurate results with reduced human intervention. In addition to improving analytical performance, the system supports resource efficiency through reduced solvent use, energy consumption, and manual handling.

Overall, ePrep ONE provides a compliant, reliable, and scalable solution that enhances workflow control while meeting the stringent regulatory and data integrity requirements of modern laboratories.

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