

ePrep ONE® | Application Note 2025

Automated Sample extraction of Polycyclic Aromatic Hydrocarbons of Liquid Paraffin

Pub No. 98-35044

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Application Benefits

- Workflow provides automated sample extraction of Polycyclic Aromatic Hydrocarbons of Liquid Paraffin
- Reduces manual handling, improves efficiency, reduces environmental impact and provides significant cost savings compared to manual extraction.

Keywords

- USP Monograph
- BP Monograph
- EP Monograph
- CFR Part 11
- Sustainability
- ePrep ONE
- Agilent Cary 3500 UV or equivalent

SUMMARY

This document describes the transition of liquid paraffin sample extraction from traditional manual liquid-liquid extraction to an automated process using the ePrep ONE Sample Extraction Workstation. The aim is to streamline the quantification of polycyclic aromatic hydrocarbons (PAHs) in liquid paraffin, ensuring compliance to European Pharmacopoeia (BP) and United States Pharmacopoeia (USP) Monographs.

Key Objectives:

- Assess the compatibility of BP (Light Liquid Paraffin) and USP (Light Mineral Oil) monographs for liquid paraffin with 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 automated method uses hexane and dimethyl sulfoxide (DMSO) for extraction and is analysed using UV-Vis spectroscopy. Several critical parameters, such as precision, linearity, and limit of detection, were evaluated. The results showed that the automated method performed as well as 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 workflow with the ePrep ONE 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 AUTOMATED LIQUID-LIQUID EXTRACTION (ALLEX)

The ALLEX (Automated Liquid-Liquid Extraction) is a revolutionary LLE technique employing a high-speed syringe to disperse solutions into micron-sized droplets, achieving near-instantaneous extraction with exceptional efficiency. Tailored for robotic workstations like the ePrep ONE, ALLEX utilizes the ePrep LongReach syringe designed for liquid-liquid extraction to ensure a sealed environment, perfect for handling volatile solvents such as hexane/DMSO. The ePrep ONE robot orchestrates the entire process with precision, positioning the ePrep LongReach syringe accurately and employing a specially designed vial to maximize extraction performance. This advanced system seamlessly manages all stages, from sample loading to autosampler vial transfer, without the need for operator intervention, even incorporating standards, pH adjustment and salting as necessary.

MATERIALS AND INSTRUMENTATION

All samples and standards were weighed as shown in Table 1, using an analytical balance (XSR104 – Mettler Toledo). They were then prepared using the fully automated sample extraction instrument, ePrep ONE. The equipment needed for performing liquid-liquid extraction on ePrep ONE is listed in Table 2.

Table 1 Reagents and Solvents required for Liquid-Liquid Extraction

Name	Grade	Brand	Mass
N-Hexane 95%	HPLC	RCI Labscan	N/A
Dimethyl Sulfoxide (DMSO)	HPLC	RCI Labscan	N/A
Naphthalene (Standard)	Analytical Grade	Chem Supply	0.07 g
2,2,4 Trimethylpentane (Iso-octane/TMP)	Analytical Grade		N/A
Liquid Paraffin (Sample)	N/A	N/A	14.3 g

Table 2 Equipment required for ePrep to perform Liquid-Liquid extractions

Glassware	Syringes/Tools	Decks
46 mL Glass High Recovery Vials	10 mL Standard Syringe	46 mL HR Vial Liquid-Liquid Rack
50 mL Glass Reagent Jars	1 mL Standard Syringe	TWD 2 x 8 x 20 mL tube Rack
20 mL Glass Vials	5 mL Probe Dispenser	Reagent Jar 5 x 50 mL
	10 mL ePrep LongReach Syringe	Vortex Mixers

Following extraction of all Samples and Standards, contaminant measurements were performed via UV/Vis Spectrophotometry using the conditions specified in the pharmacopeia monograph. For the purposes of validation, a Spectrophotometer and the UV conditions are as mentioned in Table 3.



Table 3 UV Method Conditions

Sample Reading	
Mode	Scanning
Wavelength Upper	420 nm
Wavelength Lower	260 nm
Measurement Time	3 Seconds
Path length	1 cm
Blank Solution	Washed DMSO (Using Hexane)
Standard Reading	
Mode	Fixed
Wavelength	275 nm
Path length	1 cm
Blank Solution	DMSO

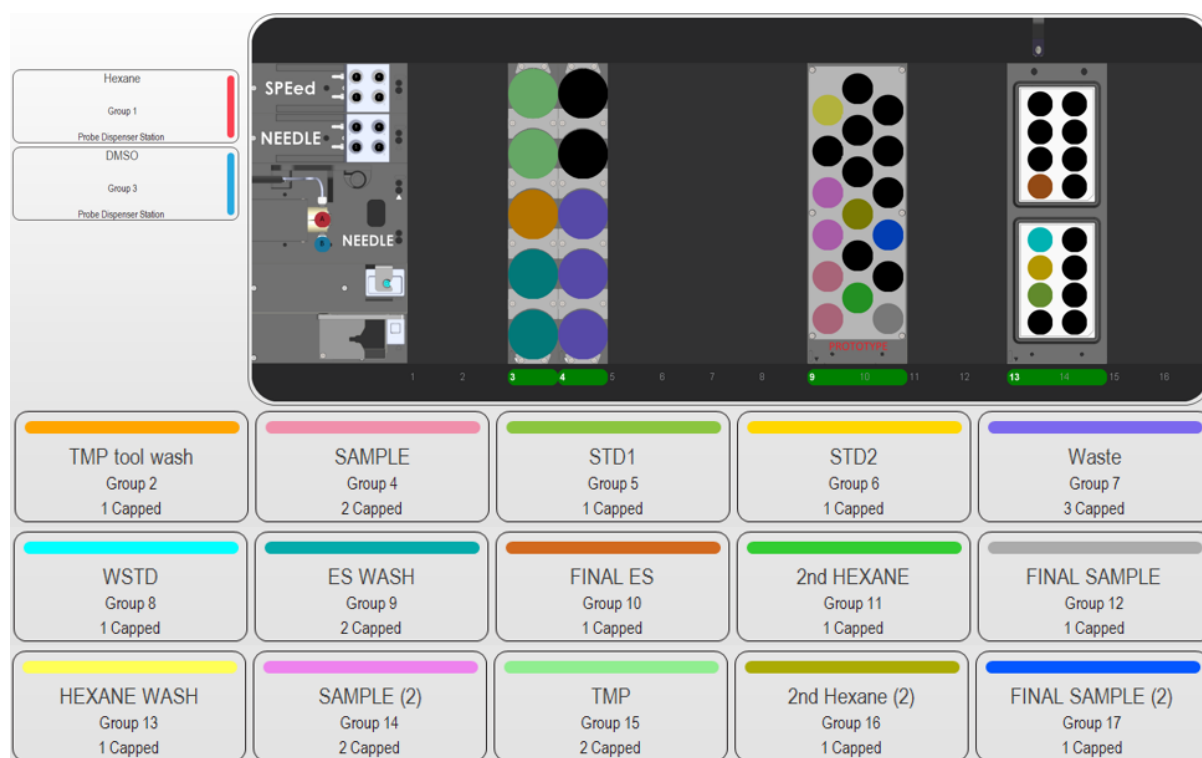


Figure 1 Screen capture of the ePrep ONE deck for 2 Liquid Paraffin samples (2 samples + 1 blank + 1 STD)



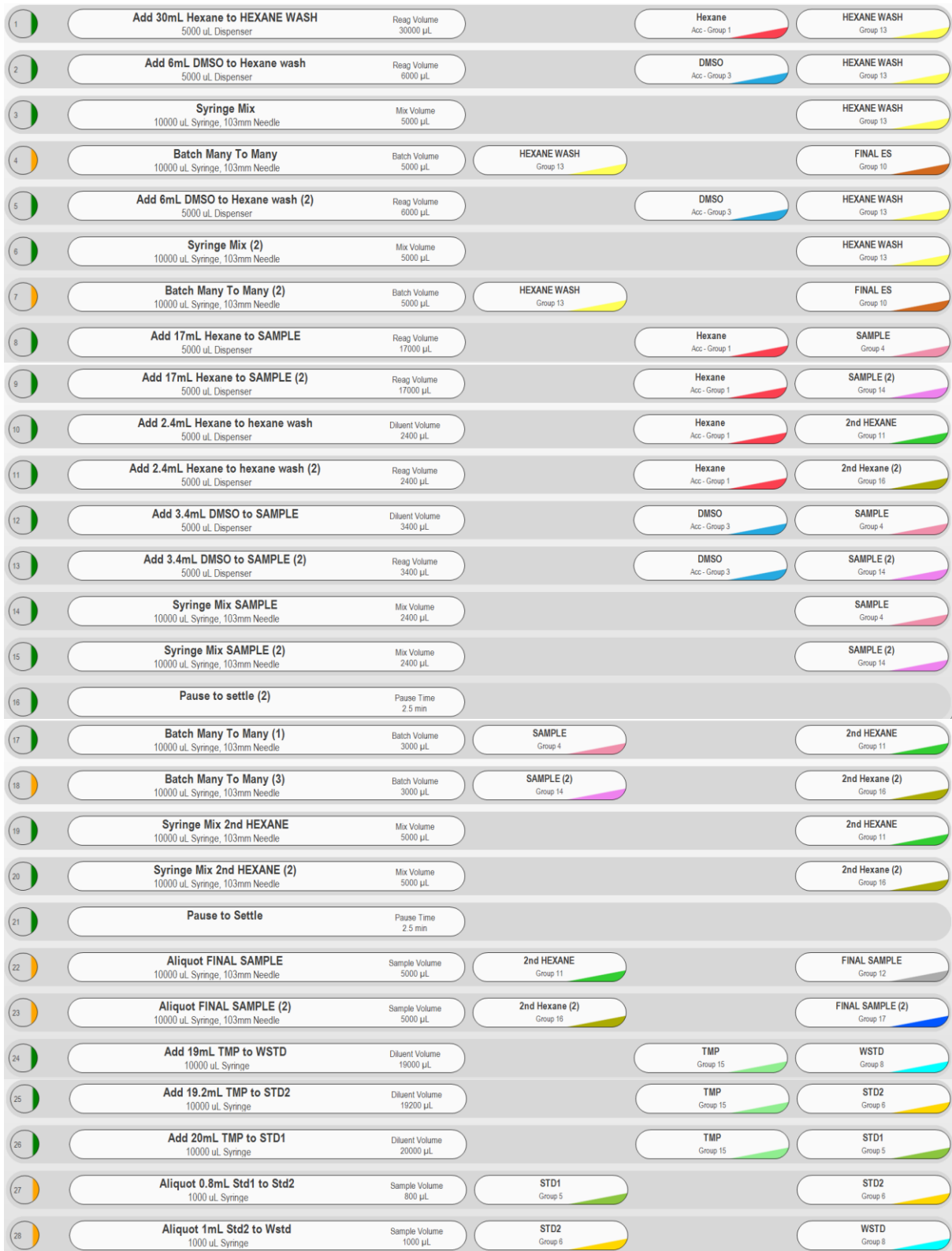


Figure 2 Screen capture of the ePrep ONE workflow

The deck setup required for the ePrep ONE workflow, and the defined groups are shown in Figure 1. The exact workflow conditions used to complete the ePrep ONE extraction is given in Figure 2.



Comparison of BP and USP Monographs to ePrep ONE

The ePrep ONE automated liquid-liquid extraction workflow 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 liquid paraffin in hexane, and subsequent extraction with DMSO, and collecting the lower layer for UV measurement.

SAMPLE EXTRACTION

In order to achieve the final extraction volume required for Good Laboratory Practice (GDP) liquid sample measurement by UV/Vis analysis using 1 cm cuvettes, duplicate samples are initially prepared for each sample replicate.

To prepare samples for examination 14.3 g of liquid paraffin is accurately weighed into a 46 mL ePrep ONE high recovery vial, this process is repeated resulting in duplicate samples. The two sample vials are then placed onto the ePrep ONE for the initial extraction into hexane. The hexane extraction is followed by precision mixing with DMSO, allowing the lower layer to be meticulously separated. A standard 20 mL ePrep ONE vial is used for both the standard and blank.

The ePrep ONE ALLEx seamlessly transfers the lower layer from the initial duplicate samples into a single second extraction vial. Where the elegant extraction process is repeated by the ePrep ONE on the combined sample without intervention of the Analyst. A diagram of the sample extraction process is given in Figure 3.

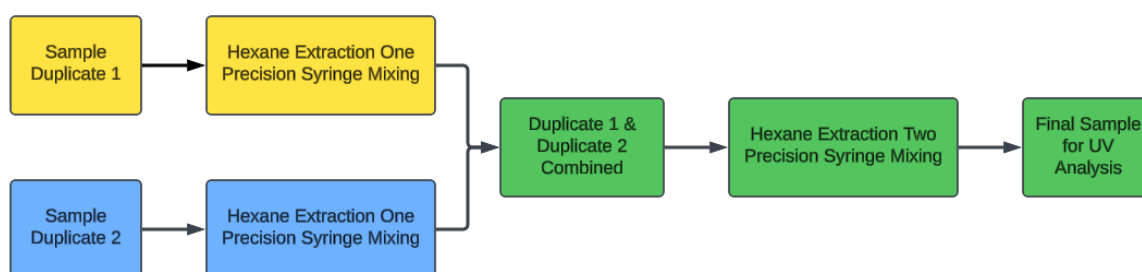


Figure 3 Simplified Process Flow of ePrep ONE Sample Extraction

The automated ePrep ONE liquid-liquid extraction maintains a 1:2 solvent ratio, replicating the conditions specified in the monograph specification while simultaneously achieving a remarkable reduction in solvent use and waste generation.

Extraction Syringe Mix Task - Parameters

The following Parameters will be discussed in detail; however, a summary of the selected parameters is in Table 4.



Table 4 Summary Table for Parameters of ePrep ONE Syringe Mix extraction

Parameter	Selected	
Vial Choice	46 mL High Recovery Glass Vials	
Needle Choice	10 mL ePrep LongReach Syringe	
Basic Settings for Long Needle Syringe:	Aspiration	Dispense Flowrate
Speed ($\mu\text{L}/\text{Sec}$)	50	400
Pause Time (Sec)	2	2
Needle Depth (mm)	Auto Low	Auto Low
Waste	Dedicated Waste Jar	
Mixing Type	Syringe Mixing	

Vial and Needle Choice

This study uses ePrep ONE 46 mL high-recovery glass vials for sample extractions, the vial selection ensures consistent results and supports efficient liquid-liquid extraction. The tapered bottom replicates the design of a separating funnel used in manual liquid-liquid extraction, facilitating clear layer separation of different liquid densities. The ePrep LongReach syringe is essential to reach the lower layer accurately, as standard needles do not support complete recovery of the lower layer following extraction.

Aspiration

Speed, Pause Time and Needle Depth: In the extraction process, a controlled aspiration speed of 50 $\mu\text{L}/\text{sec}$ was chosen to avoid air bubble formation, particularly with viscous samples. A two-second pause after aspiration allows the liquid to align with the plunger, enhancing process reliability. Combined with a low aspiration rate and Auto Low needle depth, this setup ensures robust sample extraction.

Dispersion

Speed, Pause Time and Needle Depth: For sample dispersion, less stringent parameters are needed. Dispersion speed was set to 400 $\mu\text{L}/\text{sec}$ to save time, with a two-second pause to ensure droplets fully dispense rather than sticking to the septum. Setting the needle depth to Auto Low immerses the needle into the pre-delivered solvent, preventing droplet buildup on the syringe and ensuring clean sample transfer.

Waste

Use of the wash station was deliberately avoided to prevent solvent-related damage to the tubing. Instead, a dedicated ES Wash group with DMSO was set up for needle cleaning, and a separate Waste group was created to manage the resulting waste.



Syringe Mixing

Syringe mixing was chosen to mimic the traditional manual shaking technique employed when working with separating funnels. During the initial syringe mixing step, 5.0 mL of the lower layer is aspirated and dispensed at a 35 mm height for effective interlayer mixing. The volume is ideal for creating turbulence between hexane and DMSO, promoting thorough solvent interaction. The initial syringe mixing is repeated a second time for consistency. The aspiration rate was set to 200 $\mu\text{L}/\text{sec}$, and dispense flowrate to 400 $\mu\text{L}/\text{sec}$, without pauses, this allowing some air bubbles without affecting accuracy. Following each syringe mix a settling time of 2.5 minutes is programmed into the workflow to allow the layers of solvent to settle completely before the ePrep ONE automatically continues to the next step of the workflow.

The ePrep ONE workflow combines the collected extracts from the individual samples into a single 46 mL high-recovery vial. A second syringe mixing step, where 2.4 mL of the lower layer is aspirated and dispensed at a 35 mm height is performed on the combined sample. The second mixing step is then repeated using a further 2.4 mL volume to complete the extraction of the combined sample. Once the fourth and final syringe mix is complete and the layers have been allowed to separate the ePrep ONE delivers the final sample aliquot ready for measurement.

Standard Extraction

In the ePrep ONE workflow, 0.070 g of naphthalene is weighed into a 20 mL vial, dissolved in DMSO, and vortex-mixed to create STD 1. A small aliquot of STD 1 is diluted with DMSO to make STD 2. The resulting solution is then further diluted to create the measurement standard, WSTD, ready for UV measurement. To achieve the validated concentration (0.007 mg/mL). The development team chose to utilise multiple dilution steps within the ePrep ONE workflow in place of reducing the initial standard mass. This approach was taken as it was determined that the risk of error occurring when working with a smaller standard mass was greater than the risk of error resulting from additional dilution steps. The development team would like to acknowledge that either approach to standard handling is valid and should a smaller standard mass be desired the ePrep ONE workflow can easily be adjusted to achieve this outcome.

Post Extraction Vortex Mixing Task - Parameters

The following Parameters will be discussed in detail; however a summary of the selected parameters is in Table 5

Vial and Needle Choice

For naphthalene extraction, 20 mL standard vials, a 1 mL standard needle syringe and a 10 mL standard needle syringe were used, as no special handling was needed. Despite the high concentration, the smaller vial size and use of two dilutions allowed for a dispensing volume within 10% of the syringe's capacity, meeting recommended guidelines. The decision to use the standard syringe rather than the ePrep LongReach syringe also serves to reduce the risk of sample contamination by the standard.



Aspiration

Speed, Pause Time and Needle Depth: Aspiration for both standard and sample extractions was similar, except for a one-second pause during sample extraction to allow the solvent to catch up to the plunger. This extra time was needed due to the higher viscosity of the liquid paraffin sample, which is not required for the naphthalene standard.

Table 5 Summary Table for Parameters of ePrep ONE Sample extraction

Parameter	Selected	
Vial Choice	20 mL Standard Glass Vials	
Needle Choice	10 mL Standard Needle Syringe	
	1 mL Standard Needle Syringe	
Basic Settings for 10 mL Needle Syringe:	Aspiration	Dispense Flowrate
Speed ($\mu\text{L}/\text{Sec}$)	150	400
Pause Time (Sec)	2	2
Needle Depth (mm)	Auto Low	Auto Low
Waste	Dedicated Waste Jar	
Mixing Type	Vortex Mixing	
Basic Settings for 1 mL Needle Syringe:	Aspiration	Dispense Flowrate
Speed ($\mu\text{L}/\text{Sec}$)	25	400
Pause Time (Sec)	1	1
Needle Depth (mm)	Auto Low	Auto Low
Waste	Dedicated Waste Jar	
Mixing Type	Vortex Mixing	

Dispersion

Speed, Pause Time and Needle Depth: The same can be said for the Dispersion settings, as only the pause time was changed to one second.

Waste

Dedicated waste jars which were implemented as a group, were also used for waste generated from any washes or primes.

Vortex Mixing

All standards were mixed using consistent settings: a vortex mixing time of 120 seconds at a speed of 500 RPM. This speed was crucial for generating a strong vortex, which facilitated the dissolution of Naphthalene and ensured homogeneity in both STD 2 and WSTD. By maintaining these parameters, we achieved reliable and uniform standards for our analyses.

Blank extraction

Although it is not explicitly stated in this paper, the workflow does make a Blank in the similar

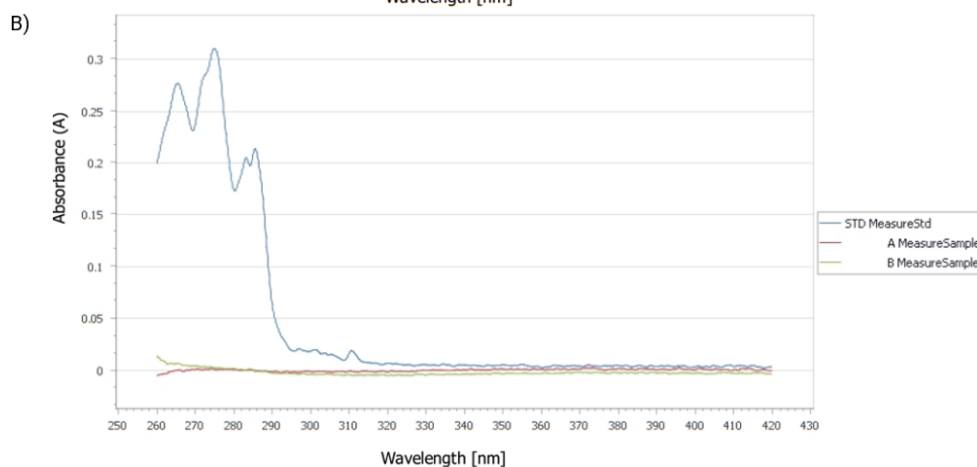
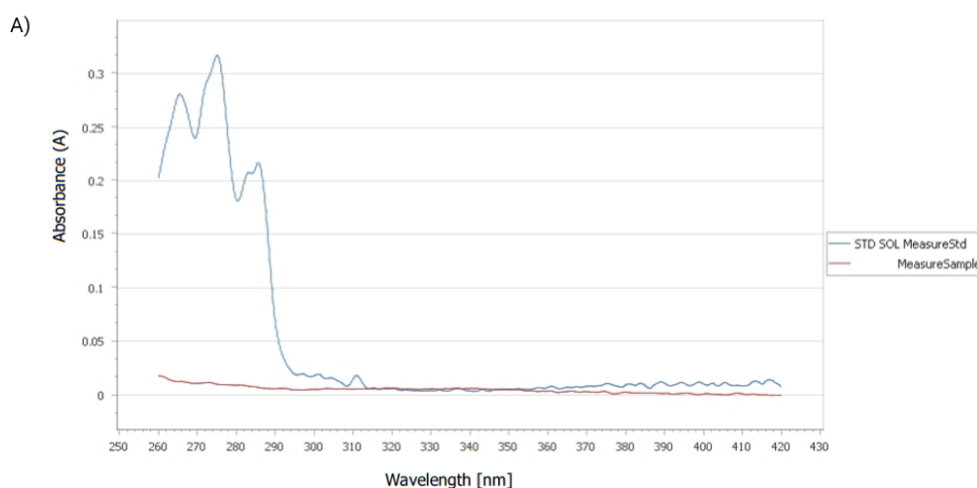


fashion as per Sample extraction where Hexane and DMSO are syringe mixed and the lower layer is collected. This workflow is shown in Figure 2.

RESULTS AND DISCUSSION

The workflow validation compares the ePrep ONE automated sample preparation method with an in-house validated approach using manual sample preparation. Two replicates of batches were prepared using the ePrep ONE and analysed via UV-Vis. The primary parameter is verifying that the liquid paraffin assay result falls within the BP 2024 limit, ensuring the sample's absorbance at 260-420 nm is no more than one-third of the standard at 275 nm. Three additional parameters were also used to assess the transfer of manual Liquid-Liquid extraction to an automated extraction using ePrep ONE:

1. Precision: Six standard extractions will be prepared, with results analysed for RSD, targeting $\leq 2.0\%$.
2. Linearity: Five standards will be prepared to create a calibration curve, with a correlation coefficient ≥ 0.980 .
3. Limit of Detection: Dilutions of the working standard will be prepared and analysed to ensure the spectrophotometer detects low concentrations.

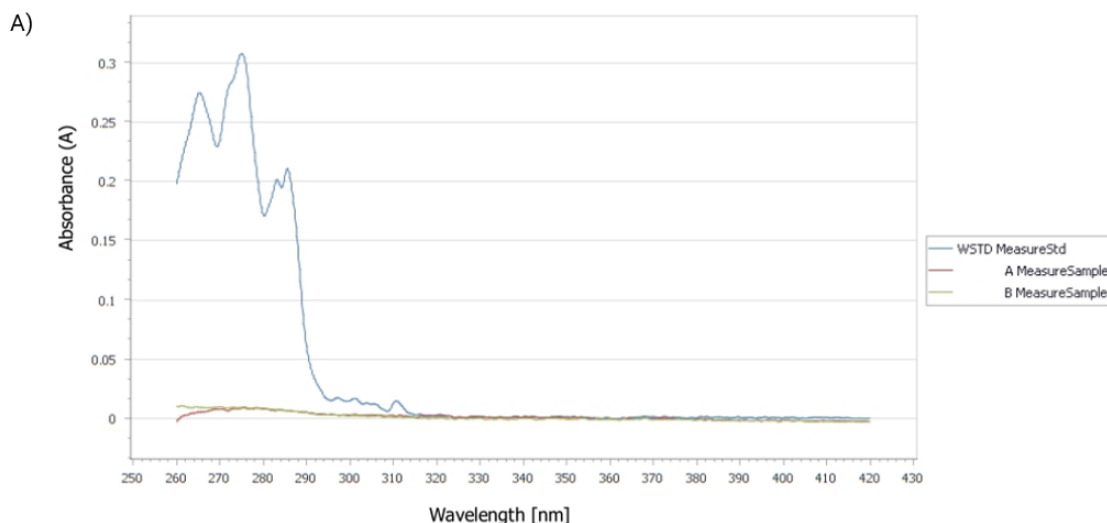


C)

Sample	Standard Absorbance	Standard Absorbance/3	ePrep ONE Absorbance	Pass/Fail	Manual Absorbance
A MeasureSample	0.3102	0.1034	0.0022	PASS	0.0176
B MeasureSample			0.0132	PASS	



Figure 4 Data acquired from the initial analyst using ePrep. A) In-House Data, B) ePrep Data, C) Tabulated Results



B)

Sample	Standard Absorbance	Standard Absorbance/3	ePrep Absorbance	Pass/Fail	Manual Absorbance
A MeasureSample	0.3080	0.1027	0.0089	PASS	0.0651
B MeasureSample			0.0106	PASS	

Figure 5 Data acquired from the second analyst using ePrep. A) ePrep Results B) Tabulated Results

Comparison of spectra from samples prepared manually and samples prepared using ePrep ONE show similar peak shapes, indicating comparable extraction results. Analyst one's ePrep extraction yielded an absorbance of ~0.0132 A, like the manually extracted sample (0.0176 A), suggesting comparable extraction efficiency, and the results were within specification. Similar results were obtained by analyst two indicating that the method is reproducible.

Table 6 RSD analysis of the absorbances at the fixed wavelength

Standards	Absorbance
1	0.30891
2	0.30498
3	0.30506
4	0.30485
5	0.30091
6	0.3650
RSD	0.86%
Extreme RSD	1.86%



This is supported by data gathered in the precision for standard extraction, where 6 standards were prepared from 1 stock and resulted in a consistent absorbance reading with RSD of 0.9% and extreme RSD of 1.9%.

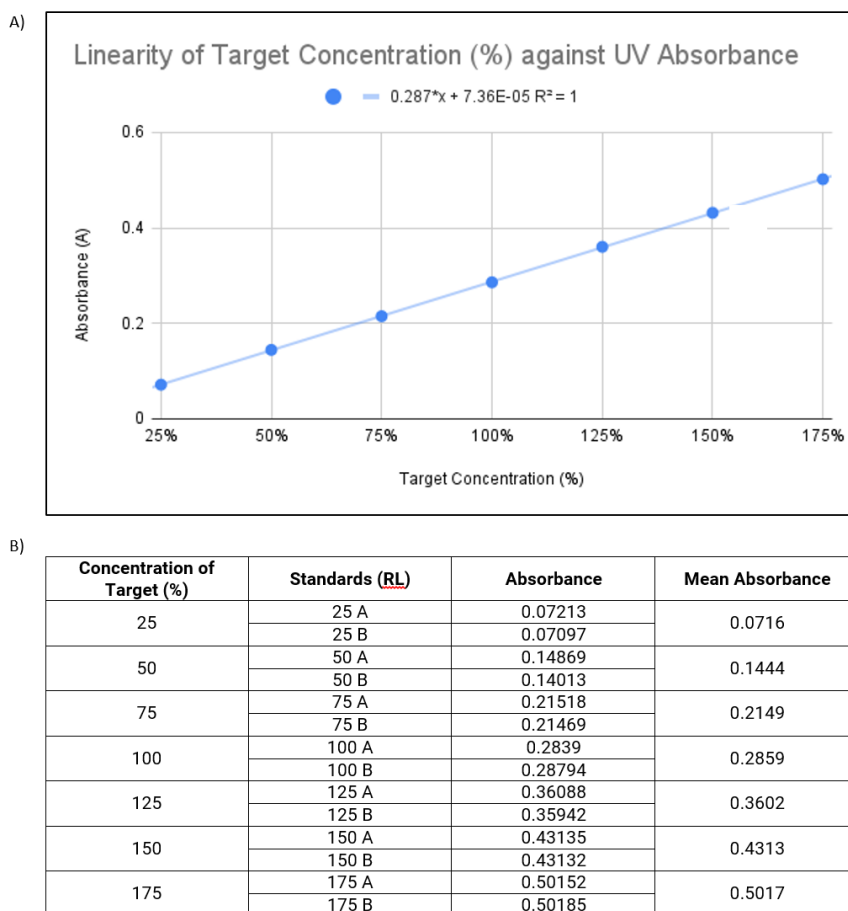


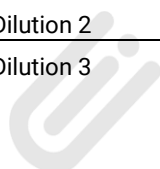
Figure 6 Linearity data with graph plotted. A) Linearity Graph B) Tabulated Data with Absorbances

Linearity was also performed with standards that were prepared from a range of 50 – 150% concentration of target. These standards were read on UV which showed an R^2 of 1.000, thus indicating that the concentrations are directly proportional to the absorbances above and below the specification as stated by BP.

Finally, a limit of detection test was performed showcasing the Stock STD being diluted multiple times with extraction solvent and then being read on the UV. Results showed that the UV will be able to read at a very low concentration indicating suitability for the limit test.

Table 7 Limit of Detection data

Sample ID	ePrep ONE Absorbance
STD 1	4.3451
STD 2	4.0764
WSTD	0.3279
Dilution 1	0.15321
Dilution 2	0.07641
Dilution 3	0.03787



Dilution 4	0.01820
Dilution 5	0.1155
Dilution 6	0.00593
Dilution 7	0.00267

CONSIDERATIONS

The analysis compares sample absorbance to a standard's absorbance ratio, using a composite sample from a single extraction, thus not reporting extraction accuracy. During development it was identified that replicating sample preparation to be within 2.0% was unobtainable due to the low level of absorbances being analysed. Nevertheless, the standard deviation and relative standard deviation (RSD) of automated ePrep ONE extraction surpassed results obtained from manually extracted samples in precision.

While ePrep ONE presents higher absorbance readings than those obtained from manual extraction samples, determining extraction efficiency remains complex. These elevated absorbance levels are viewed as a worst-case scenario for this test. Furthermore, the method transfer for liquid paraffin excludes washed hexane, as washing extended workflow time and led to abnormal results. Tests revealed no significant difference between washed and unwashed hexane, highlighting the streamlined efficiency of the process. During development it was observed that the DMSO used for sample extraction is critical, the DMSO used for extraction must be freshly prepared and protected from light.

BENEFITS

Automation in sample extraction offers significant financial, environmental, and human benefits. The ePrep ONE system, with its 21 CFR Part 11 software, ensures compliance by providing a validated workflow equivalent to pharmacopeia standards, meeting strict quality and regulatory requirements. The ePrep ONE automates the extraction process, reducing human intervention and reducing risk of errors while delivering exceptional precision and accuracy. By following the guidelines of the British Pharmacopoeia (BP), European Pharmacopoeia (EP), and United States Pharmacopeia (USP) Monographs, the ePrep ONE enhances operational efficiency and aligns laboratory practices with regulatory standards, ensuring data integrity and reliability.

The ePrep ONE system can transform laboratory practices by optimizing reagent use, minimizing waste, and reducing hazardous chemical disposal, thus achieving sustainability goals and decreasing the ecological footprint. For analysts, it frees them from repetitive tasks, improving job satisfaction and allowing them to focus on more complex scientific work. It also enhances safety by reducing exposure to hazardous substances and minimizing lost time due to repetitive stress injuries.

In summary, automated sample extraction with ePrep ONE boosts efficiency, sustainability, and workplace safety, greatly benefiting both the laboratory environment and its professionals. Delivering Quality to Customers first time every time.



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Table 8 Comparison of manual extraction to ePrep extractions

Comparison	Manual extraction	ePrep extraction
Solvent Usage	628 mL	123 mL
Duration	2 Hours	2 Hours
Hands on Time	2 Hours	10 Minutes

CONCLUSION

The shift from manual liquid-liquid extraction to the automated ePrep ONE for liquid paraffin marks a significant advancement in efficiency, solvent conservation, and precision. The automated ePrep ONE automated workflow not only complies with the stringent standards of the United States Pharmacopeia (USP), British Pharmacopoeia (BP), and European Pharmacopoeia (EP) monographs but also matches the performance of traditional manual methods. Rigorous validation confirms the ePrep ONE's ability to easily deliver precise sample preparation with minimal human intervention, enhancing consistency and reducing errors. The study highlights the substantial savings in time and resources, particularly through decreased solvent use and labour achieved using the ePrep ONE - essential for laboratories seeking to improve operational efficiency.

Automated sample preparation with the ePrep ONE signifies a new era of scientific progress. The ePrep ONE delivers Quality laboratory analysis consistently, reducing risks associated with manual sample preparation, optimizing laboratory throughput, and promoting sustainability by minimizing solvent use and chemical waste generation. Adopting automated workflows with the ePrep ONE empowers laboratories to achieve exceptional productivity while maintaining global quality standards.

APPENDIX 1

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

Description	Part Number
ePrep ONE Sample Preparation Workstation Kit	01-01000
ePrep Probe Dispenser	01-04300
ePrep Vortex Mixer, 2P	01-04100
Reagent Jar Adapter Plate supplied with 5 X50 ml Reagent Jars x 2	01-03085
Adapter Plate, 2x4 Ø28mm Vials, Microtiter Format	01-03081
Rack Only, 2 x 4 Ø28mm Vials, Microtiter Format x 2	01-03092
46mL High Recovery Vials (Pk 100)	01-18000
Septa Caps for 46mL HR Vials (Pk 100)	01-18001
Adaptor Plate 2P, 46mL HR Vials	01-03148
10mL LongReach Syringe	01-09037

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