Application

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

ePrep ONE® | Application Note 2025

Automated Sample extraction of Polycyclic Aromatic Hydrocarbons of Petroleum Jelly

Pub No. 98-35042

Authors

Ego Pharmaceuticals:
H. Evans-Lemmo, J. Lacomel
Monash University:
A.Viney, S. Ly
ePrep:
A.Minett

Keywords

- USP Monograph
- BP Monograph
- CFR Part 11
- Sustainability
- ePrep ONE
- Mettler UV/Vis

Application Benefits

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

SUMMARY

This document describes the transition of petroleum jelly 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 petroleum jelly, ensuring compliance with British Pharmacopoeia (BP) and United States Pharmacopeia (USP) Monographs.

Key Objectives:

- Assess the compatibility of BP (White Soft Paraffin) and USP (Petrolatum) monographs for petroleum jelly 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 contact with 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 ONE 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 an analytical glass syringe that ensures a sealed environment, perfect for handling volatile solvents such as hexane/DMSO. The ePrep ONE robot orchestrates the entire process with precision, positioning the syringe accurately and employing a specially designed container 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 using 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	0.06 g
Petroleum Jelly (Samples)	N/A	N/A	0.5 g

Table 2 Equipment required for ePrep ONE Liquid-Liquid extractions

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

Following extraction of all Samples and Standards, contaminent 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	265 nm
Measurement Time	3 Seconds
Path length	1 cm
Blank Solution	Washed DMSO (Using Hexane)
Standard Reading	
Mode	Fixed
Wavelength	278
Path length	1 cm
Blank Solution	DMSO

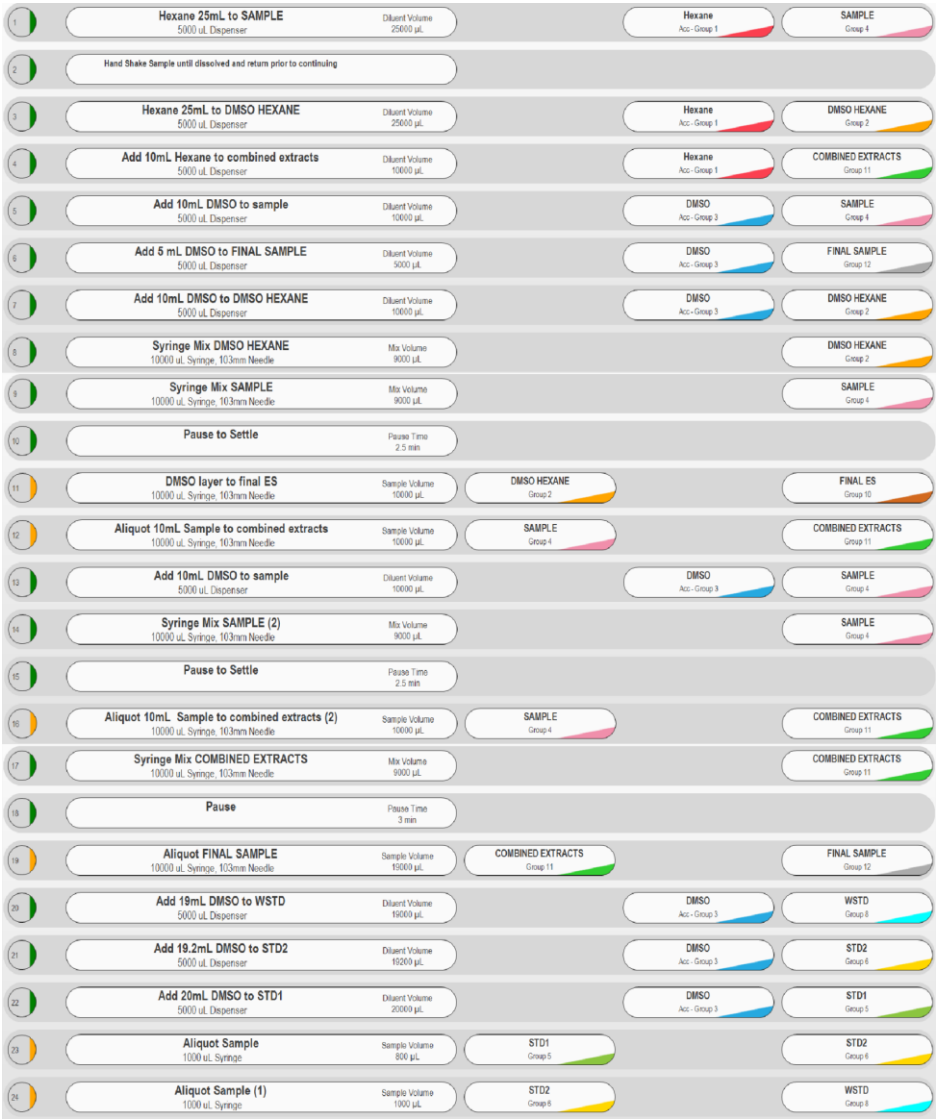


Figure 1 Screen capture of the ePrep ONE workflow

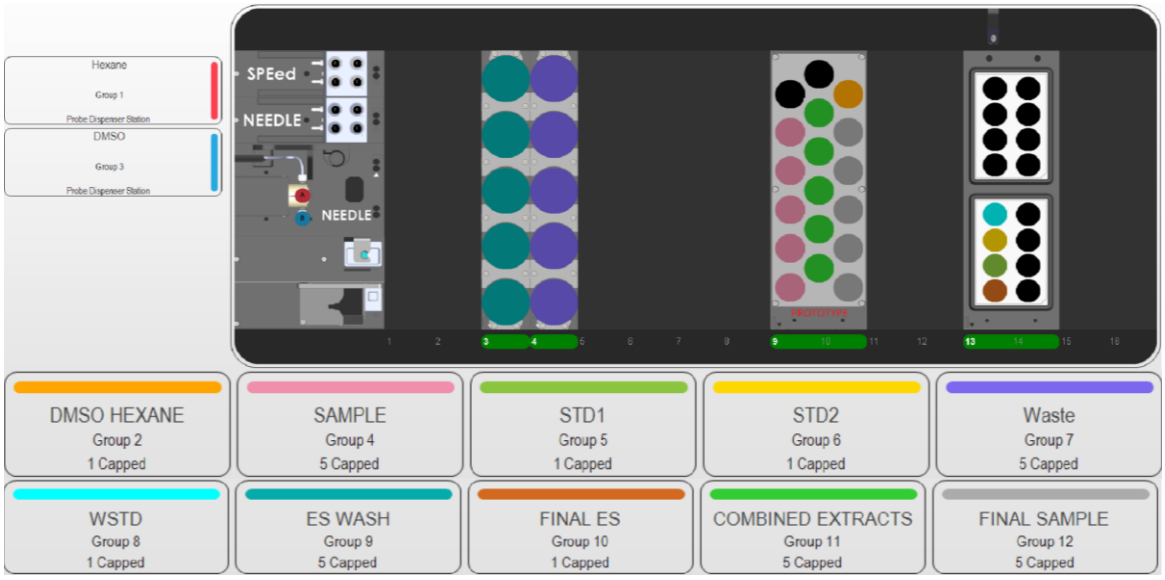


Figure 2 Screen capture of the ePrep ONE deck for 5 Petroleum Jelly samples (5 samples + 1 blank + 1 STD)

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

Comparison of BP and USP Monographs to ePrep ONE automated workflow

The ePrep ONE workflow is compared to an in-house validated Ego Pharmacuetical 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 dissolving petroleum jelly in hexane, extracting with DMSO, and collecting the lower layer for UV measurement.

SAMPLE EXTRACTION

In the automated ePrep ONE workflow, the petroleum jelly sample undergoes extraction into hexane, followed by precision mixing with DMSO, allowing the lower layer to be meticulously separated into a second vial. The elegant extraction process is repeated by the instrument without intervention of the Analyst with an additional hexane mix. The workflow culminates in the collection of the final extract for UV/Vis analysis. The automated ePrep ONE workflow 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 Syringe Mix extraction

Parameter	Selected	
Vial Choice	46 mL High Recovery Glass Vials	
Needle Choice	10 mL Long Needle Syringe	
Basic Settings for Long Needle Syringe:	Aspiration	Dispersion
Speed (µL/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 the high-recovery glass vials for extractions, the glass vials ensure consistent results and support efficient extraction. Their tapered bottom replicates the separating funnel used in manual extraction, allowing clear layer separation for different liquid densities. A long-needle syringe was essential to reach the bottom layer accurately, as standard needles were too short for this design.

Aspiration

Speed, Pause Time and Needle Depth: In the extraction process, a controlled aspiration speed of 50 µL/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 µL/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 it in the pre-delivered solvent, preventing droplet buildup on the syringe and ensuring clean sample transfer.

Waste

The wash station was 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 shaking method, with 9.0 mL of the lower layer aspirated and dispensed at a 45 mm height for effective interlayer mixing. This volume

was ideal for creating turbulence between hexane and DMSO, promoting thorough solvent interaction. Mixing was repeated three times for consistency. The aspiration rate was set to 150 µL/sec, and dispersion to 500 µL/sec, without pauses, this allowing some air bubbles without affecting accuracy.

Standard Extraction

In the ePrep ONE workflow, 0.060 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, which is then further diluted to create WSTD, ready for measurement. To achieve the validated concentration (0.006 mg/mL) while accommodating smaller ePrep ONE volumes, multiple dilutions were used instead of directly adjusting the initial weight, making routine handling easier for analysts.

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

Table 1 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	
Basic Settings for Long Needle Syringe:	Aspiration	Dispersion
Speed (µL/Sec)	50	400
Pause Time (Sec)	1	1
Needle Depth (mm)	Auto Low	Auto Low
Waste	Dedicated Waste Jar	
Mixing Type	Vortex Mixing	

Vial and Needle Choice

For naphthalene extraction, 20 mL standard vials 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.

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 petroleum jelly sample, unlike the naphthalene standard.

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.

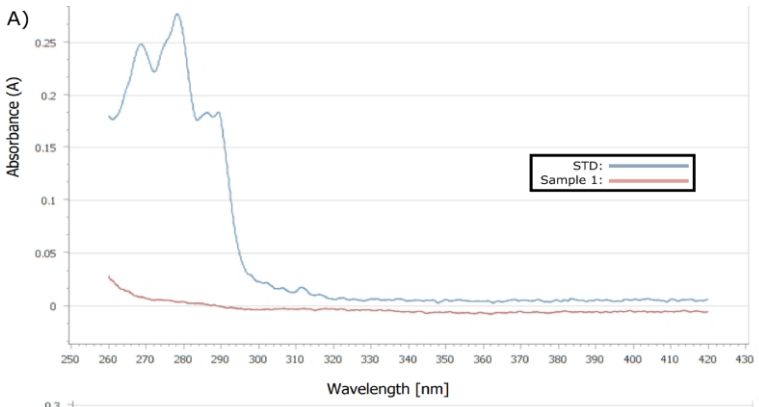
Blank extraction

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 7.

RESULTS AND DISCUSSION

The workflow validation compares the ePrep ONE automated extraction with an in-house validated approach. Two replicates of batches will be prepared using ePrep ONE and analysed via UV-Vis. The primary parameter is verifying that the petroleum jelly assay result falls within the BP 2024 limit, ensuring the sample's absorbance at 265-420 nm is no more than one-quarter of the standard at 278 nm. Three parameters will also assess the transfer of conventional Liquid-Liquid extraction to ePrep ONE:

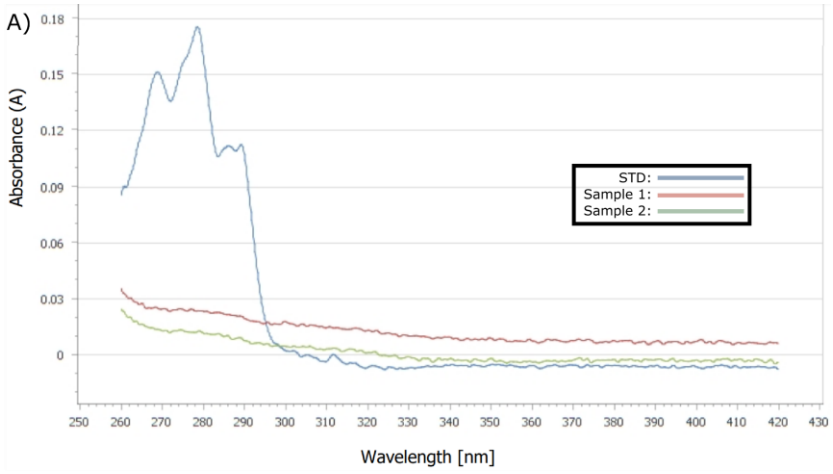
1. Precision: Six standard extractions will be prepared, with results analysed for RSD, targeting ≤ 2.0%.
2. Linearity: Five standards will be prepared to create a calibration curve, with a correlation coefficient ≥ 0.980.
3. Limit of Detection: Dilutions of the working standard will be prepared and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



C)

Sample	Standard Absorbance	Standard Absorbance/4	ePrep Absorbance	Pass/Fail	Manual Absorbance
1	0.2859	0.07145	0.0255	PASS	0.02515
2			0.0383	PASS	

Figure 3 Data acquired from the initial analyst using ePrep ONE. A) In-House Data, B) ePrep ONE Data, C) Tabulated Results



B)

Sample	Standard Absorbance	Standard Absorbance/4	ePrep Absorbance	Pass/Fail	Manual Absorbance
1	0.1745	0.04362	0.0352	PASS	0.02515
2			0.0241	PASS	

Figure 4 Data acquired from the second analyst using ePrep ONE. A) ePrep ONE Results B) Tabulated Results

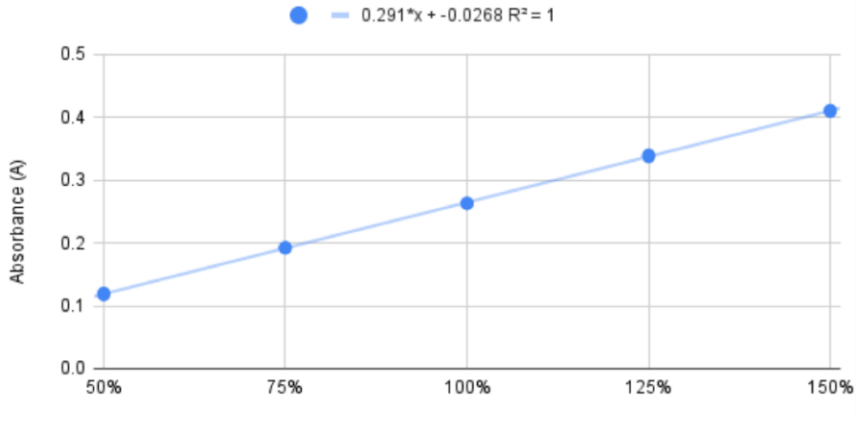
Comparison of spectra from manual and ePrep ONE sample extraction shows similar peak shapes, indicating comparable extraction results. Analyst one's ePrep ONE extraction yielded an absorbance of ~0.0319 A, like the manually extracted sample (0.02515 A), suggesting comparable extraction efficiency, and the results were within specification. However, when analyst two performed the extraction using the same batch, the STD absorbance was lower, though the sample results still passed. The difference was attributed to a 3-minute variation in mixing time during analyst two's workflow.

Table 6 RSD analysis of the absorbances at the fixed wavelength

Standards	Absorbance
1	0.2745
2	0.2741
3	0.2700
4	0.2717
5	0.2682
6	0.2724
RSD	0.89%
Extreme RSD	1.64%

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 1.6%.

A) Linearity of Target Concentration (%) against UV Absorbance



B)

Concentration of Target (%)	Standards	Absorbance	Mean Absorbance
50	50 A	0.1202	0.119
	50 B	0.1178	
75	75 A	0.1924	0.192
	75 B	0.1923	
100	100 A	0.263	0.263
	100 B	0.263	
125	125 A	0.3374	0.3386
	125 B	0.3398	
150	150 A	0.4108	0.4102
	150 B	0.4096	

Figure 5 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.

Table 2 Limit of Detection data

Sample ID	ePrep ONE Absorbance
STD 1	3.9289
STD 2	3.9289
WSTD	0.2724
Dilution 1	0.1227
Dilution 2	0.0481
Dilution 3	0.0143
Dilution 4	-0.0037
Dilution 5	-0.00179

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.

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, achieving extraction replication within 2.0% was challenging due to low absorbance levels. Nevertheless, the standard deviation and relative standard deviation (RSD) 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 petroleum jelly 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.

BENEFITS

Automation in sample extraction heralds transformative financial, environmental, and human advantages. In addition, the ePrep ONE's 21 CRF Part 11 software ensures compliance, by providing a validated pharmacopeia-equivalent workflow 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. By adhering to the guidelines of the British Pharmacopoeia (BP), European Pharmacopoeia (EP) and United States Pharmacopeia (USP) Monographs, 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 hazardous chemical disposal, 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.

Table 8 Comparison of manual extraction to ePrep ONE extractions

Comparison	Manual extraction	ePrep extraction
Solvent Usage	945 ml	150 ml
Duration	2 Hours	1 Hour
Hands on Time	2 Hours	10 Minutes

CONCLUSION

The transition from manual liquid-liquid extraction to the automated ePrep ONE for petroleum jelly exemplifies a transformative leap in efficiency, solvent conservation, and precision. This automated workflow not only meets the stringent specifications of the United States Pharmacopeia (USP), British Pharmacopoeia (BP), and European Pharmacopoeia (EP) monographs, but also rivals the performance of traditional manual methods. Rigorous validation has affirmed the ePrep ONE's prowess in delivering precise sample preparation, significantly reducing human intervention and thereby enhancing consistency and minimizing errors. The study underscores remarkable savings in both time and resources, notably through reduced solvent usage and labour.

This method emerges as an indispensable tool for laboratories aiming to elevate operational efficiency, particularly in anticipation of the upcoming USP mandatory requirement for polycyclic aromatic hydrocarbons (PAHs) in petroleum jelly.

In essence, automation of sample preparation using the ePrep ONE heralds a new era of scientific advancement. The ePrep ONE offers profound benefits by diminishing human error, optimizing laboratory throughput, and championing sustainability through decreased solvent use and generation of chemical waste. Embracing automated workflows, achieved using the ePrep ONE as demonstrated here, empowers laboratories to achieve unparalleled productivity while steadfastly adhering to global Quality standards.