Maximizing Analyte Recovery using Novum™ Simplified Liquid Extraction (SLE) 96-Well Plates

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Introduction

Supported Liquid Extraction (SLE) has been developed as an alternative sample preparation method to remedy some of the drawbacks of liquid-liquid extraction (LLE) including the formation of emulsions, the use of large amounts of hazardous solvents, and difficulty in automating. Traditional SLE implements a solid support of diatomaceous earth that absorbs an aqueous-based sample to increase the surface area between it and the extracting solvent, yielding an extremely efficient liquid-liquid extraction with no emulsions and no need to manually separate liquids. Although efficient, traditional diatomaceous earth-based SLE products can be prone to sorbent inconsistencies and limited availability due to the fact that diatomaceous earth is a natural resource. Recent advances have introduced Novum Simplified Liquid Extraction (SLE) products which are packed with a unique, synthetic SLE sorbent that alleviates the challenges associated with a natural resource. The novel, synthetic Novum SLE sorbent also provides additional benefits including a higher loading capacity and consistently more in-well headroom. For example, Novum MAX 96-well plates have a loading capacity of 450 µL and a headroom of 1.15 mL while the Biotage® Isolute® SLE+ 400 µL 96-well plate has a headroom of 1.05 mL.

In an effort to transition methods away from traditional diatomaceous earth SLE materials, we performed a case study on a steroid panel with a specific focus on betamethasone to demonstrate two effective ways to increase recovery using the novel Novum synthetic SLE material.

Materials and Methods

All reagents and solvents were HPLC or analytical grade. Analyses were performed using an API 3000™ LC/MS/MS (AB SCIEX, Framingham, MA).

Sample Preparation

200 µL Load:

1. 100 µL of plasma was diluted with 100 µL of water
2. Load 200 µL diluted (1:1) water:plasma or spiked plasma into each well of a Novum SLE MINI 96-Well Plate (part no. 8E-S138-FGA)
3. Apply 5” Hg for 10 seconds
4. Wait for five minutes
5. Load 1x 1 mL elution solvent and elute by gravity flow
6. Apply 5” Hg vacuum for 10 seconds to complete elution
7. Blow down with N_2 (40 °C for 60 minutes). Initially at 12 psi (for 20 minutes), then at 30 psi
8. Reconstitute with 100 µL Acetonitrile/Water (20:80) by vortexing the plate at 1200 rpm for 5 minutes

300 µL Load:

1. 100 µL of plasma was diluted with 200 µL of water
2. Load 300 µL diluted (2:1) water:plasma or spiked plasma into each well of a Novum SLE MINI 96-Well Plate (part no. 8E-S138-FGA)
3. Apply 5” Hg for 10 seconds
4. Wait for five minutes
5. Load 1x 1 mL elution solvent and elute by gravity flow
6. Apply 5” Hg vacuum for 10 seconds to complete elution
7. Blow down with N_2 (40 °C for 60 minutes). Initially at 12 psi (for 20 minutes), then at 30 psi
8. Reconstitute with 100 µL Acetonitrile/Water (20:80) by vortexing the plate at 1200 rpm for 5 minutes

HPLC Conditions

HPLC analysis was performed with a Kinetex® 2.6 µm C18, 50 x 2.0 mm column packed with core-shell particle media, providing high resolving power and fast analysis time.

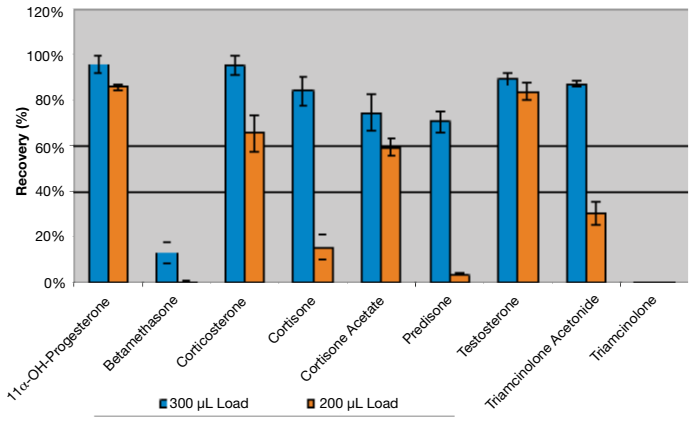
Column:	Kinetex 2.6 µm C18
Dimensions:	50 x 2.0 mm
Part No.:	00B-4622-AN
Mobile Phase:	A: Water with 0.1 % Formic acid B: Acetonitrile with 0.1 % Formic acid
Flow Rate:	0.4 mL/min
Gradient:	**Time (min) B (%)** 0.00 20 3.00 95 3.50 95 3.51 20 6.00 20
Injection Volume:	10 µL
Temperature:	Ambient
Detection:	API 3000™ MS/MS (AB SCIEX)

Results and Discussion

Maximizing Recoveries by Diluting to the Maximum Aqueous Holding Capacity of the Plate

Figure 1 compares recoveries of 9 steroids from plasma using two different loading volumes on the Novum™ SLE MINI 96-well plate while using dichloromentane (DCM) as the extraction solvent. It was determined that loading a total volume of 300 µL (100 µL plasma plus 200 µL water) produced higher recoveries as compared to loading a total volume of 200 µL. By diluting a sample to the total water holding capacity of the plate (300 µL on the Novum SLE MINI plate and 450 µL on the Novum SLE MAX plate), there is a higher surface area for interaction with the extraction solvent, improving the rate at which analytes partition into the organic elution solvent. A similar effect can be achieved when using the Novum SLE MAX 96-well plate by diluting the sample to the plate's full aqueous holding capacity of 450 µL.

Figure 1. Analyte Recoveries under Various Loading Volumes



Maximizing Recoveries Using a Binary Solvent System

Another way to increase analyte recovery using the Novum SLE plate is to implement a binary solvent system using ethyl acetate. **Figure 2** compares the recoveries between an extraction using DCM, EtOAc, and 10 % ethyl acetate (EtOAc) in DCM. While the EtOAc provides the best recoveries for the steroid panel, **Figure 2** shows the marked improvement in recovery of betamethasone that can be obtained by adding 10 % EtOAc to the DCM extraction (optimized method in **Table 1**). This trend is consistent across other extraction solvents as well, indicating that an improvement in recovery can be achieved by adding 5-10 % EtOAc to any water immiscible extraction solvent (including but not limited to Hexane, DCM, MTBE, etc.).

While we were able to improve recovery by adding 10 % EtOAc to our DCM elution, Triamcinolone still displayed poor recovery under both the original and the optimized methods. Because Triamcinolone is very polar (Log P ~0.25), it simply requires a more polar extraction solvent to remove it from the aqueous sample, which is why only EtOAc gives acceptable recovery.

Figure 2. Elution Solvent Optimization Study

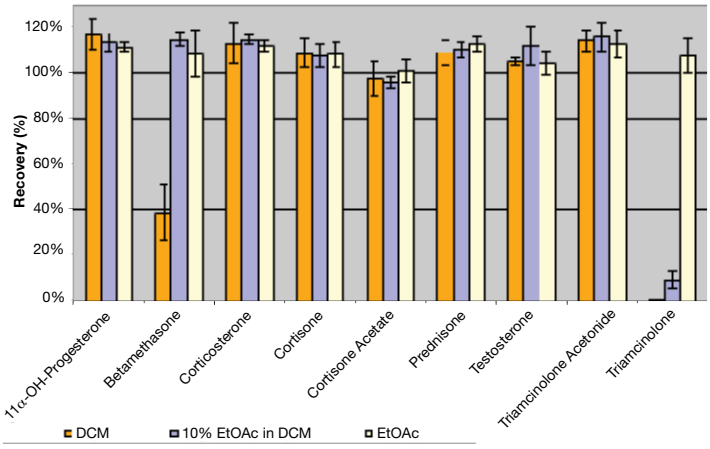


Table 1. Final Optimized Method

Novum SLE Optimized DCM Extraction (Novum SLE MINI 96-well plate, Part No. 8E-S138-FGA)
1. Dilute 100 µL plasma with 200 µL water
2. Load 300 µL of sample onto Novum SLE MINI 96-Well Plate
3. Apply 5" Hg of vacuum for 10 seconds
4. Wait for five minutes
5. Add 1 mL DCM:EtOAc (90:10) and elute by gravity
6. Apply 5" Hg for 10 seconds to complete elution
7. Blow down with N_2 (40 °C for 60 minutes). Initially at 12 psi (for 20 minutes), then at 30 psi.
8. Reconstitute with 100 µL Acetonitrile/Water (20:80) by vortexing the plate at 1200 rpm for 5 minutes

Conclusion

This work shows the broad versatility of Novum SLE 96-well plates which are able to implement a variety of extraction solvents depending on analyst and analyte preference. By performing a case study using betamethasone from plasma, we present helpful optimization tips to boost analyte recovery with Novum SLE 96-well plates. While using ethyl acetate as an extraction solvent provides the best recoveries in most cases, this study shows that diluting the sample up to a total volume of 300 µL on the Novum SLE MINI 96-well plate and 450 µL on the Novum SLE MAX 96-well plate will maximize the area of partition between the aqueous sample and the organic solvent, thus improving recovery. Moreover, adding 5-10 % ethyl acetate to any organic extraction solvent will improve its ability to wet the sorbent which also yields improved recovery. However with very polar compounds, it may be necessary to use 100 % ethyl acetate as an extraction solvent to obtain acceptable recoveries.

Ordering Information

Novum™ Simplified Liquid Extraction (SLE)

Part No.	Description	Unit/Box
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/Box

Accessories

Collection Plates (deep well, polypropylene)		Unit
AH0-7192	96-Well Collection Plate, 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate, 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate, 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
Sealing Mats		
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk
Vacuum Manifold		
AH0-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea

guarantee

If Novum SLE products do not perform as well or better than your current SLE product, return the product with your comparative data within 45 days for a FULL REFUND.

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