APPLICATIONS

Novum™ Simplified Liquid Extraction (SLE) Method Development – A Corticosteroid Case Study

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Introduction

Simplified Liquid Extraction (SLE), also known as Supported Liquid Extraction, has been developed as an alternative sample preparation method to liquid-liquid extraction (LLE) to remedy some of the drawbacks including the formation of emulsions, difficulty in automating, and the use of large amounts of hazardous solvents. The particle size of the solid support in an SLE material mimics the shaking event in a LLE workflow and creates micrometer scale containers for aqueous sample. By increasing the surface area and reducing the diffusion distances, one promotes a rapid and efficient extraction that negates the necessity of manual phase separation. Novum, a synthetic SLE sorbent, yields results that are identical in most cases to contemporary diatomaceous earth SLE products, but is not restricted by limitations that are associated with natural resources such as availability and variation in composition. In this technical note we will investigate a case study using a corticosteroid panel to demonstrate that Novum is capable of achieving equivalent recoveries, while also offering the analyst the added option of additional clean up and phospholipid removal that cannot be achieved on traditional diatomaceous earth SLE products. We also offer general tips that can help with the method development process for other compounds of interest.

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

Novum SLE 12 cc Tube, part no. 8B-S138-KDG

Pretreatment

Three samples were prepared for extraction, each contained 1800 µL diluted (1:1) plasma or spiked plasma plus A, B, or C (below)

A. + 0 µL water
B. + 200 µL water
C. + 400 µL water

SLE Procedure

1. Load sample onto the Novum SLE sorbent
2. Apply 5” Hg vacuum for 10 seconds
3. Wait for 5 minutes
4. Apply 2x 5 mL DCM and allow to elute under gravity
5. Apply 5” Hg vacuum for 10 seconds to complete elution

Add 10 µL IS solution to each extract except the blank sample. Blow down with N_2 (room temperature for 60 minutes). Reconstitute with 200 µL ACN/Water (20:80) by vortexing the tube at 1000 rpm for 2 minutes.

HPLC Conditions

Column:	Kinetex® 2.6 µm C18
Dimensions:	50 x 2.1 mm
Part No.:	00B-4462-AN
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Acetonitrile
Gradient:	**Time (min) B (%)** 0.00 20 0.01 20 3.00 95 3.50 95 3.51 20 8.00 20
Flow Rate:	400 µL/min
Injection:	5 µL
Temperature:	30 °C
Detection:	MS/MS, API 3000™ (AB SCIEX)
System:	Agilent® 1260 Binary Pump

Figure 1. Phospholipid breakthrough as a result of sample load volume

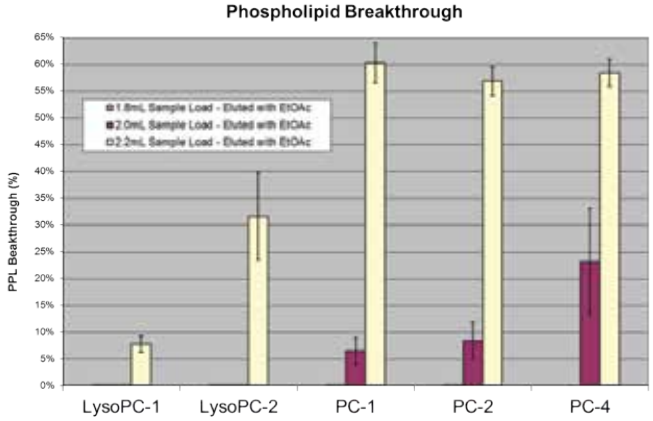
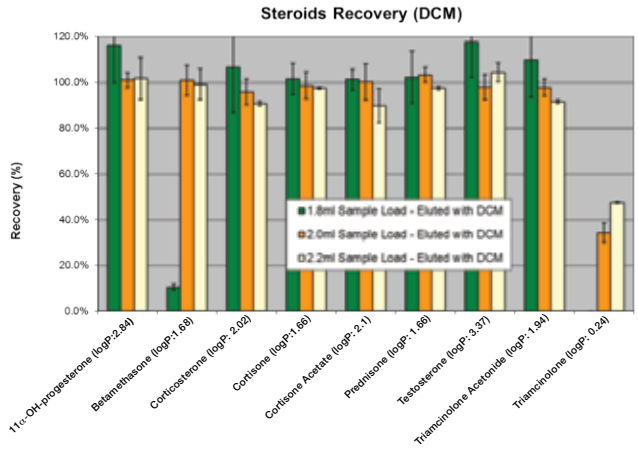


Figure 2. Analyte recovery as a result of sample load volume

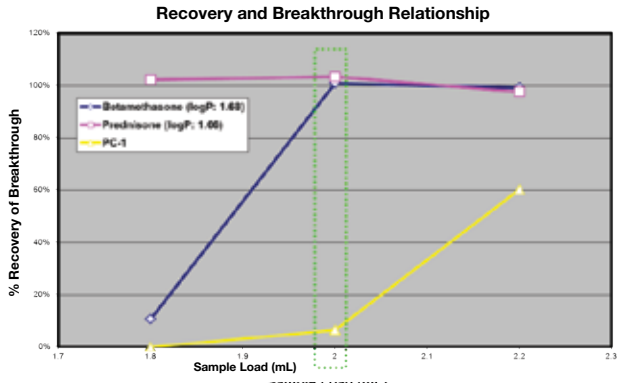


Results and Discussion

Cleanliness

In the development of the Novum SLE 12 cc tube, a total aqueous holding capacity of 2 mL was targeted. The first part of this investigation began with a look at the phospholipid breakthrough as a representation of sample cleanliness. This study was performed using ethyl acetate as the organic elution solvent. What

Figure 3. Phospholipid breakthrough vs. analyte recovery as a result of sample load volume



was noticed was that at 1.8 mL sample loading volume, there was no breakthrough of any phospholipids. At a 2 mL sample load volume, there was a slight increase in the breakthrough of Phosphotidylcholines (PC-1, PC-2, and PC-4), but not Lysophosphotidylcholines (LysoPC-1 and LysoPC-2). At 2.2 mL, there was a significant breakthrough of both Phosphotidylcholines and Lysophosphotidylcholines. The difference and quantity in phospholipid breakthrough can be explained by two different phenomenons which are explained below.

By overloading the cartridge beyond the 2.0 mL capacity, there is a literal breakthrough of the aqueous sample into the collection plate prior to addition of the organic solvent, which yields phospholipid levels that are drastically higher in comparison to the 2 mL load. This breakthrough also includes Lysophosphotidylcholines that were not present at the 2 mL load.

By staying either at or below the aqueous holding capacity, one can manipulate the cleanliness of the sample. By underloading the sorbent (as seen in **Figure 1** in the case of 1.8 mL), one decreases the surface area of interaction between the sample and the extraction solvent, limiting the partition of phospholipids from the aqueous to the extraction solvent by increasing the diffusion distance required to partition. By the same logic, loading the tube to the full aqueous holding capacity increases the surface area of interaction between the sample and extracting solvent which decreases the diffusion distance required for the phospholipids (and analytes). At full aqueous holding capacity, the synthetic sorbent results in high recoveries of target compounds and a limited partitioning of phospholipids that is analogous to traditional diatomaceous earth SLE material.

Recovery

Another factor in determining a build for the Novum™ SLE 12 cc tube was an evaluation of recovery for a class of compounds. In **Figure 2** the varying recoveries are examined for nine compounds in a corticosteroid suite. Notice that the recovery of triamcinolone is poor at all aqueous loads; this is due to its polar nature (LogP ~0.24) and should be expected when extracting with a non-polar solvent like DCM. To improve recovery of this compound it is necessary to extract with a more polar water immiscible solvent like ethyl acetate.

The focal point of this data lies in the recovery of betamethasone. Notice that at the 1.8 mL load, betamethasone gives poor recovery of < 20 %. By increasing the aqueous load of the sample to 2.0 mL, recovery was improved up to nearly 100 %. There is also no added benefit for recovery by loading >2.0 mL. This is the same phenomenon that was mentioned in the Cleanliness section. By increasing the amount of aqueous sample that is loaded onto the sorbent, the surface area of the sample is increased which facilitates a faster, more efficient partitioning of betamethasone into the extraction solvent, thus drastically improving recovery.

This was somewhat to be expected as the Novum synthetic SLE material is much more hydrophilic than traditional diatomaceous earth SLE material. Because betamethasone is a fluorinated compound, one might expect to see some added H-bonding with Novum's surface that is overcome by increasing the amount of aqueous sample loaded onto the sorbent, thus increasing surface area and decreasing the partitioning distance into the extraction solvent.

Notice, however, that the recovery of seven of the nine compounds in this suite was greater than 80 % even at the 1.8 mL loading volume.

Putting it all together – a Balance Between Cleanliness and Recovery

By combining the data from both the phospholipid depletion experiment (**Figure 1**) as well as the recovery study (**Figure 2**) we are able to create **Figure 3**, which shows a very obvious "sweet spot" for the recovery of both betamethasone and prednisone. This "sweet spot" limits the amount of phospholipid partitioning while maximizing recovery. By loading the plate at its full aqueous holding capacity (2 mL), we maximize the surface area which decreases the partition path into the extraction solvent, resulting in higher recoveries of both betamethasone and prednisone. However, if you omit betamethasone from **Figure 3**, the optimal loading conditions are found at 1.8 mL because loading to 2.0 mL does not provide any additional increase in recovery for prednisone however it does result in a slight breakthrough of phospholipids. So, if one has a set of easily eluted compounds such as prednisone and the six other compounds found in **Figure 2**, loading at 2 mL provides no additional recovery benefit and actually provides a dirtier sample making a 1.8 mL load ideal.

With that said, Novum SLE provides the additional benefit that traditional diatomaceous earth SLE does not; an ability to tune the cleanliness of the sample by varying the aqueous sample loaded onto the sorbent. And as seen in the case of prednisone (**Figure 3**), the additional surface area is sometimes not necessary and a smaller load volume can be applied to selectively eliminate phospholipids while maintaining acceptable recovery.

Conclusion

In this work we showed that by varying the aqueous loading volume onto the synthetic Novum SLE sorbent, it is possible to manipulate the cleanliness and the recovery of the sample. Depending on the analytes being extracted, there is often a "sweet spot" for both cleanup and recovery. By loading the sorbent to its full aqueous holding capacity, you minimize the distance of diffusion between all constituents in the matrix and the organic solvent, which will in effect maximize your recovery, while also facilitating a slight partition on phospholipids (more specifically Phosphotidylcholines) into your extraction solvent. By decreasing the aqueous load, we show that it is possible to completely eliminate interferences such as phospholipids while maintaining high recoveries for certain, if not most compounds. Moreover, based on the knowledge that this material is much more hydrophilic than diatomaceous earth material, we can show that for compounds like fluorinated betamethasone (and other compounds that might H-bond with the hydrophilic nature of the Novum sorbent), it is necessary to load to the total aqueous loading capacity of the sorbent in order to maintain acceptable recoveries. In conclusion, it is important to determine the proper sample loading volume for your particular analytes to determine the "sweet spot" that provides both high recoveries and adequate cleanliness.

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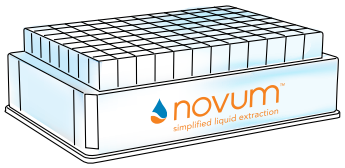
Ordering Information

Novum™ Simplified Liquid Extraction (SLE) 96-Well Plates



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

96-Well Plate Accessories

Part No.	Description	Unit
Collection Plates (deep well, polypropylene)		
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/well Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL/well 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

Novum SLE Tubes

Part No.	Description	Unit
8B-S138-FAK	Novum SLE 1 cc tubes	100/pk
8B-S138-5BJ	Novum SLE 3 cc tubes	50/pk
8B-S138-JCH	Novum SLE 6 cc tubes	30/pk
8B-S138-KDG	Novum SLE 12 cc tubes	20/pk

Tube Accessories

Vacuum Manifolds		
Part No.	**Description**	**Unit**
AH0-6023	12-Position Vacuum Manifold Set	ea
AH0-6024	24-Position Vacuum Manifold Set	ea

guarantee

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

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