Cleanert®
Bio-scavenger

Your Unique Choice!

Fast, Simple & Effective
Protein Precipitation / Phospholipid removal

- Elimination of ion suppression effects due to phospholipids
  - Benefit of improved MS sensitivity
  - Benefit of more reliable and consistent data
- Less LC/MS downtime
- Longer HPLC column lifetimes

Agela Technologies®

www.agela.com

Labs worldwide are using PPT (Protein Precipitation) and Phospholipid Removal Plates

Biological samples contain a large number of phospholipids (amphoteric molecules) and the presence of these phospholipids often causes serious matrix effects (especially when using LC/MS). Protein precipitation on its own has very limited purification ability so this cannot be a reliable process to remove phospholipids.

Once phospholipids enter the LC/MS system, they can accumulate on the HPLC column and at the ion source. This can seriously affect the lifespan of the column as well as leading to ion suppression effects for analytes of interest. More seriously, the retention times of the peaks associated with the accumulated phospholipids can be unpredictable, worsening as the analysis proceeds.

Although every effort can be made to avoid co-elution of phospholipids and analytes during method development, there is still a risk of actual analysis failure.

Benefits of Bio-scavenger Protein and Phospholipid removal plates:

- Extremely simplified sample preparation process
- 99% phospholipids removal simultaneous with the protein removal
- Stable batch to batch reproducibility
- Extends HPLC column lifetime by reducing sample contamination
- Significantly reduced sample retest rate

Simplified sample preparation suitable for acidic, basic and neutral compounds.

Plasma/Serum

↓

Four times volume of acidified ACN

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Sample Loading: 50-200 µL

↓

Mixing: Repeated suction or vortex mixing thoroughly

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Collection: Positive Pressure/ Negative Pressure.

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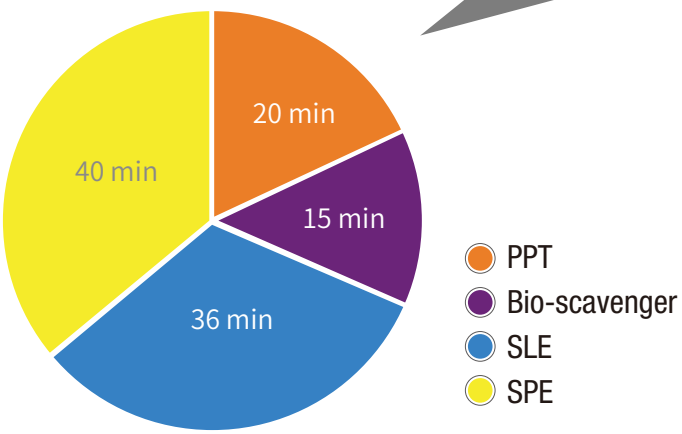
Analysis: LC-MS/MS

15 minutes sample processing time using Bio-scavenger*, up to 25% faster than PPT AND removes phospho-lipids.

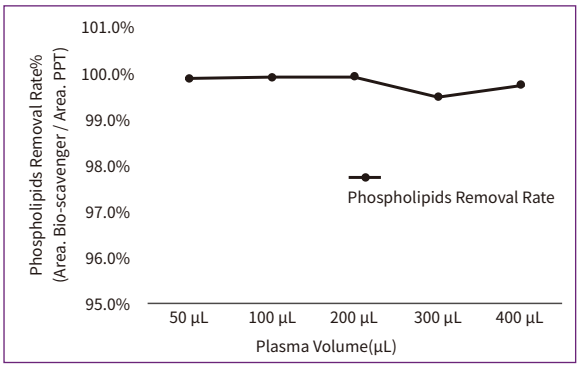


* Considering nitrogen blow down time, the time of SLE and SPE may vary.

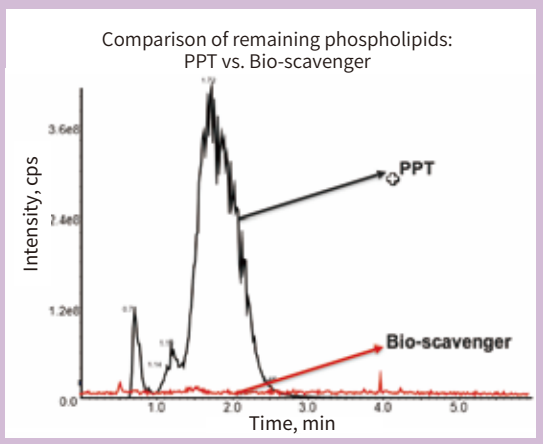
Bio-scavenger can remove up to 99% of phospholipids in bio-samples.

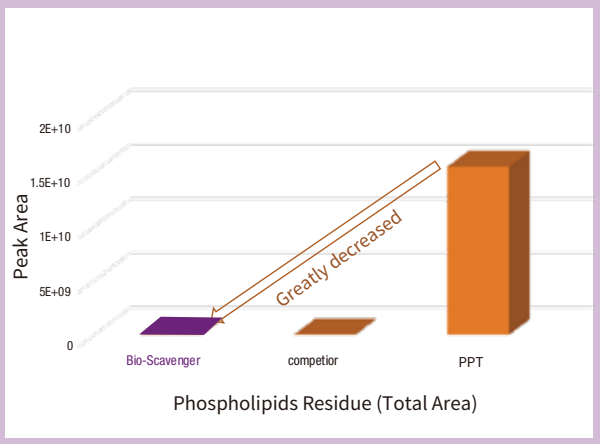
We compared between 50-400mL sampling volume (184/184 Channel Testing used).

Bio-scavenger gives a consistant phospholipids removal rate.

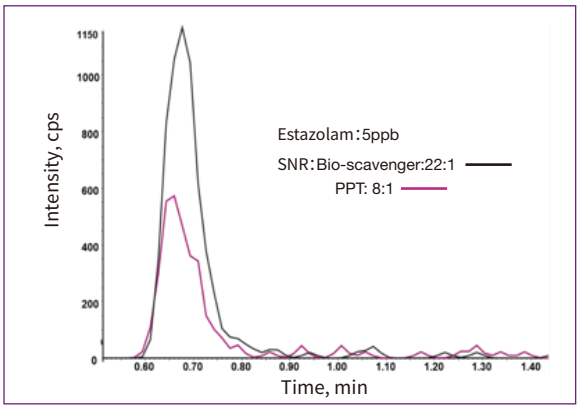


This advantage is more obvious when compare with PPT:

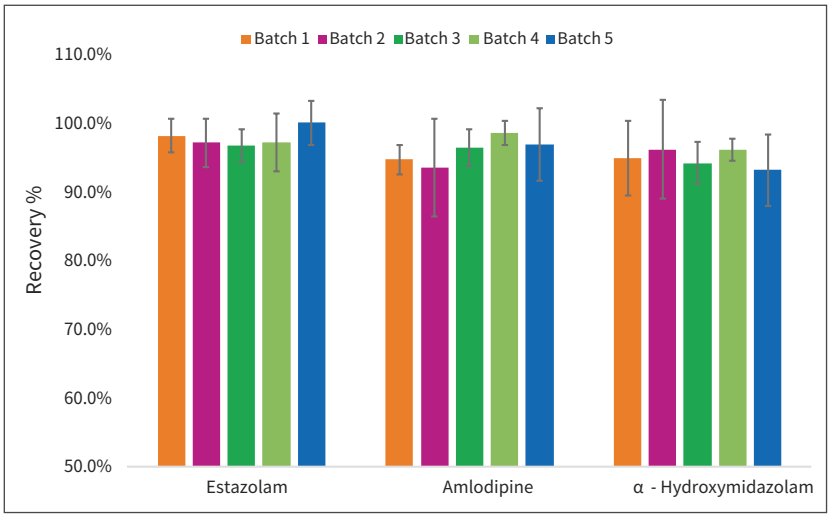




Now, let us see the performance of Cleanert® Bio-scavenger during actual analysis: Removal of phospholipids can significantly reduce matrix effect.



Batch to Batch reproducibility of Bio-scavenger.



The recoveries of 3 typical compounds (acidic, basic and neutral) from 5 batches were tested, the data indicates that there is no significant differences between different batches, and RSD is less than 10%.

During continuous injections, the samples processed by Cleanert® Bio-scavenger showed good stability. This resolves the discrepancy between apparent QC passing rate (consistent Ratio value), and oversized internal standard compound RSD response.

In addition, phospholipids can accumulate in column over time, which can cause reduced MS response and shorten column lifetime. HPLC columns analyzing samples prepared with PPT plates have phospholipids accumulated under long-term use, resulting in reduced MS signal.

Cleanert Bio-scavenger Ordering Guide:

Product Name	Specs	UOM	P/N
Cleanert Bio-scavenger 96-well plate	30 mg/2 mL/well	2/pk	Bio-0302W
Cleanert Bio-scavenger cartridge	30 mg/1 mL	100/pk	Bio-0301

Quick Q&As for usage of Bio-scavenger:

1

Q: How to choose a proper precipitation reagent when analyzing plasma or serum matrices?

A: Acetonitrile with 0.1% formic acid is universally used as the first option. And considering the solubility of compounds, we can also try Acetonitrile with 1% formic acid : Methanol (90:10, V/V).

2

Q: Can ammonia acetonitrile solution be used for precipitation?

A: Although the presence of ammonia does not significantly reduce the removal of phospholipids, we do not recommend the use of ammonia acetonitrile solution for extraction to ensure the robustness when analyzing large quantities of samples.

3

Q: How should I handle trace amount samples, e.g. 20 µL?

A: This can be done by increasing the amount of precipitant. For example, you could try adding 10 times or more acetonitrile used for extraction. One principle is that the total volume should be greater than 200 µL.

4

Q: What should I do if I find that the recovery rate of the sample is not as good as protein precipitation?

A: Bio-scavenger has excellent purification ability to remove phospholipids without considering the acidity and alkalinity of the target analytes. On rare occasion, some hydrophobic compounds may be lost due to adsorption issues. In this scenario, please consider secondary extraction, increasing the proportion of organic phase or using our Mas-C series phospholipid removal products.

5

Q: If the plate becomes blocked or it is difficult for liquid to pass through, what should be done?

A: First, increase the pressure to extract under the constant flow rate (3-5 seconds a drop) . In addition, we recommend that acetonitrile and sample be added vertically (even against the sieve plate) and thoroughly oscillate (ensure that the liquid is suspended) to completely precipitate the protein.

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