MRL Boston

# Strategies for reducing phospholipid-based matrix effects in LC-ESI-MS bioanalysis

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# Outline

- Matrix effect
  - Impact of matrix effect
  - Causes of matrix effect
  - Detection of matrix effect
- Phospholipid-based matrix effect
- Sample preparation strategies

# Impact of matrix effect

Two major effects are associated with matrix effect:

- Influence of ion suppression on data quality
- Unwanted matrix build up causing downtime

**Data quality**

- Poor accuracy and sensitivity which lead to unreliable recovery data
  - Matrix components or new/unexpected metabolites can co-elute with analyte and affect ionization
  - MS response is altered by presence of matrix ions
  - Ion suppression leads to poor limits of detection/quantification
- Irreproducibility which leads to poor method robustness
  - Degree of effect can differ among sources of plasma or other matrices
  - Can lead to divergent standard curves
  - Different plasma samples will exhibit different ion suppression/enhancement

**Downtime**

- MS downtime
  - Build up of lipids/proteins/salts in MS source
    - MS source pollution
- Column lifetime
  - Build up of lipids/proteins on-column
    - Increase in back-pressure over time

# Matrix effect

Matrix effects are any undesired observed effects caused by the presence of residual matrix components in the sample which co-elute with the analyte of interest

The presence of residual matrix components after sample pretreatment can alter the MS response

- Loss of signal (Ion suppression)
- Gain in signal (Ion enhancement)

Phospholipids identified as a major source of matrix effects in plasma

Other plasma components (e.g. proteins), formulation agents, mobile phase modifiers, phthalates and plasticizers from plasticware and concomitant co-eluting analytes (metabolites or other co-medication) may also contribute

Extremely analyte specific

# Causes of matrix effect

Matrix effects are due to co-eluting residual matrix components or other chemicals and can change:

- droplet surface tension
  - larger droplets and insufficient desolvation
- ion evaporation selectivity
  - surfactants can gather at droplet surface and preferentially go through ion evaporation
- mass of the analyte ion
  - by ion-pairing and adduct formation

or any matrix components which can cause:

- Co-precipitation of analyte with non-volatile matrix components
- Gas phase deprotonation

# Detection of matrix effect

Two approaches can be used to detect matrix effect:

- Comparison of analyte peak area in neat solution to analyte peak area spiked into extracted blank matrix
  - Indicates the presence and effect of interference, but does not give chromatographic profile of interference (i.e. where in the chromatogram it occurs)
  - Measures the extent of matrix effect
    - *% Matrix Effect = (A - B)/B x 100*

    Where, B is the peak area of a neat standard and A is the corresponding peak area for standards spiked into blank matrix after extraction
- Post-column infusion of analyte
  - Shows chromatographic profile of interference, but does not directly measure the extent of matrix effect on analyte

# Phospholipids-based matrix effect

Phospholipids can compete with the analyte and influence:

- ionization in MS sources
- desolvation of the LC effluent droplets

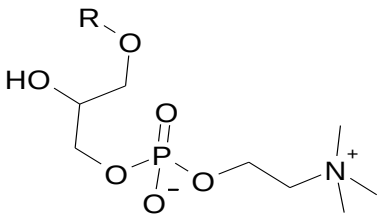
Their molecular structures exhibit two major functional groups:

- polar head group which is zwitterionic and remains charged from high to low pH
- tail with one or two long chain fatty acid ester groups, which impart considerable hydrophobicity to the molecule

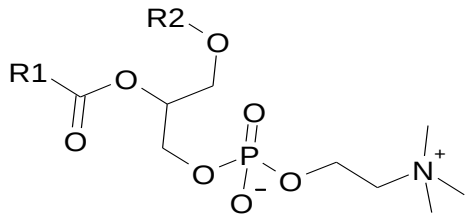
Phospholipids-based matrix effect is difficult to predict and control

- plasma variability
- inter-subject variability (diet, protein levels, etc.)
- species variability
- dependent on concentration of lipids

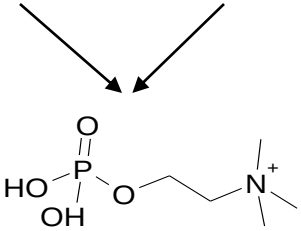
# Phospholipids-based matrix effect (cont)




Lysophosphatidylcholines




Glycerophosphocholines




Ion fragment m/z 184

| Lysophosphatidylcholines | Glycerophosphocholines |
|---|---|
| m/z 496→m/z 184 | m/z 704→m/z 184 |
| m/z 524→m/z 184 | m/z 758→m/z 184 |
| | m/z 806→m/z 184 |

# Detection of phospholipids-based matrix effect

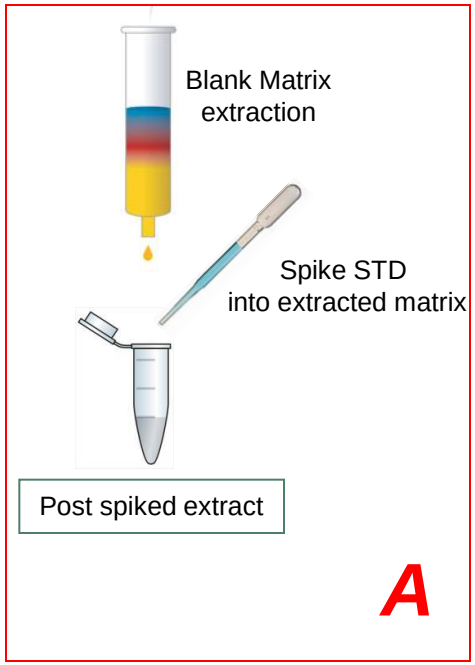
Inject the same amount of a neat standard solution and a post spiked extract, making sure they are in the same solvent.

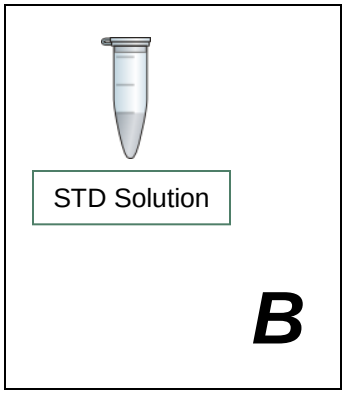
Matrix Effect can then be quantified:

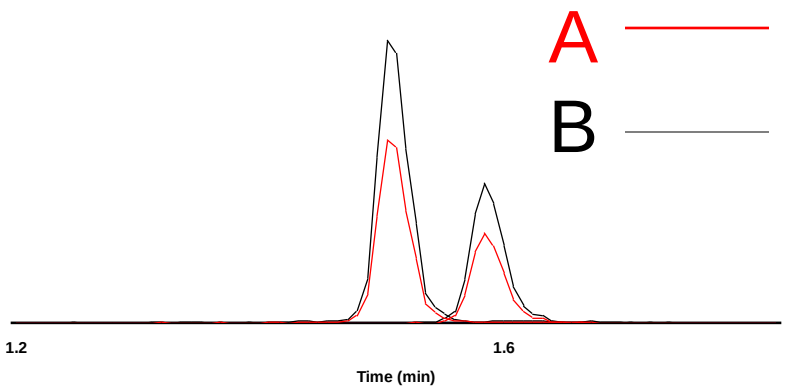
*% Matrix Effect = (A - B)/B x 100*

**A**: is the peak area of a standard spiked into blank matrix after extraction

**B**: is the peak area of a neat standard

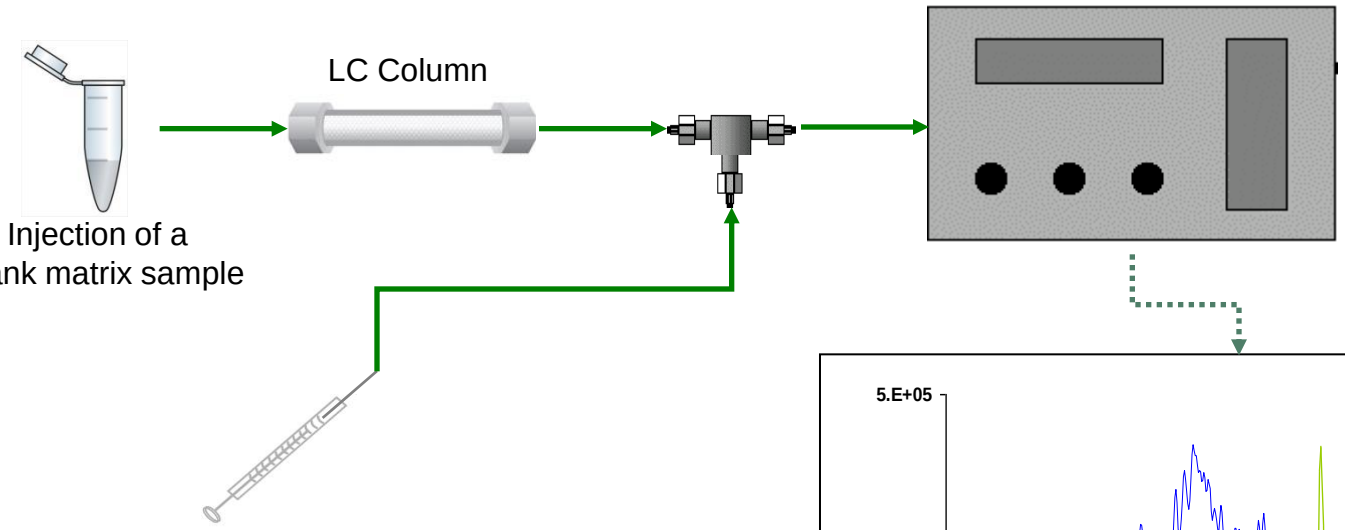


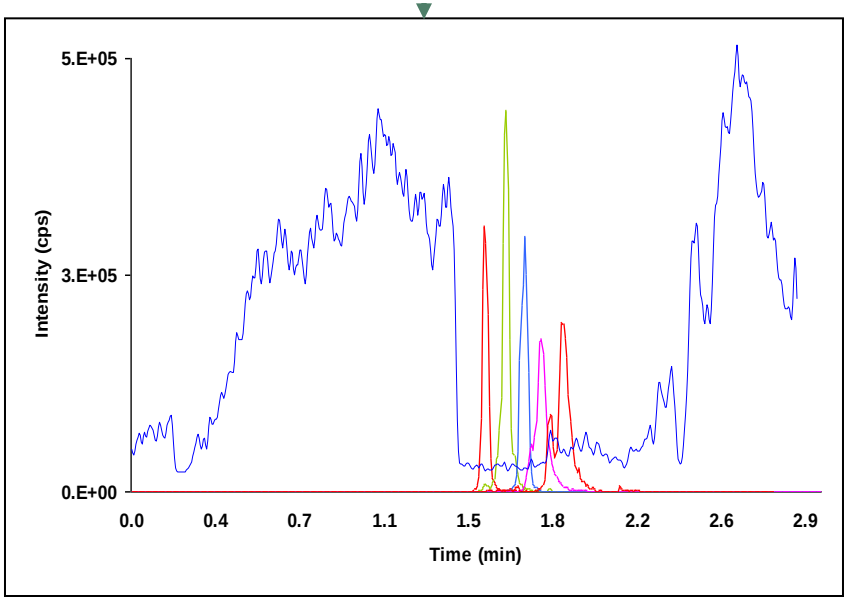



# Detection of phospholipids-based matrix effect (cont)

Post-column infusion is a way to show ion-suppression. An injection of blank matrix is made into a constant infusion of the analyte:




Continuous infusion of analyte with syringe pump




Negative dips in trace are indicative of ion suppression while peaks are due to standard phospholipds

# Sample preparation strategies

Several techniques have been applied toward the removal of phospholipids in order to obtain cleaner sample extracts

- Dilution
  *dilutes interferences*
- Protein Precipitation (PPT) with colloidal silica
  *removes protein and lipids*
- Solid Phase Extraction (SPE)
  *potentially removes proteins, lipids, salts and others*
- Liquid/Liquid Extraction (LLE) or Supported Liquid Extraction
  *potentially removes proteins, lipids, salts and others*

- Selective depletion of lipids using of 96-well filtration plates
  *effectively removes proteins and lipids*
  HybridSPE-PPT® (Supelco / Sigma-Aldrich)
  Captiva® (Varian)
  Ostro® (Waters)

# Sample preparation strategies (cont)

Single step LLE and SPE methods or either followed by other clean-up steps can guarantee high extraction yields and clean extracts.

- On-line SPE
  - on-line end-capped phenyl SPE resulted in reduced sample preparation time and cleaner extracts
    - *J. Mass Spectrom. 33 (1998) 1071*
- SPE using mixed-mode cation exchange sorbents
  - removes up to >99% of phospholipids relative to PPT
    - *J. Chromatogr. B 859 (2007) 84*
    - *J. Pharm. Biomed. Anal. 37 (2005) 359*
    - *J. Am. Soc. Mass Spectrom. 14 (2003) 1290*
- PPT using colloidal silica agents
  - the treatment of plasma with a colloidal silica suspension and lanthanum chloride followed by PPT with acetonitrile can selectively remove plasma phospholipids
    - *Rapid Commun. Mass Spectrom. 22 (2008) 2873*
- LLE and cerium modified columns
  - cerium-modified column and methyl-tert-butyl-ether (MTBE) liquid–liquid extraction were employed to remove phospholipids from serum
    - *Metabolomics, 2 (2006) 145*
- Supported liquid extraction (SLE)
  - using Isolute SLE+ 200 mg plate and traditional extraction solvents SLE and LLE provided comparable removal of phospholipids
    - *O.A. Ismaiel et al J. Chromatogr. B (2010) in press*

# Sample preparation strategies (cont)

SPE and LLE advantages:

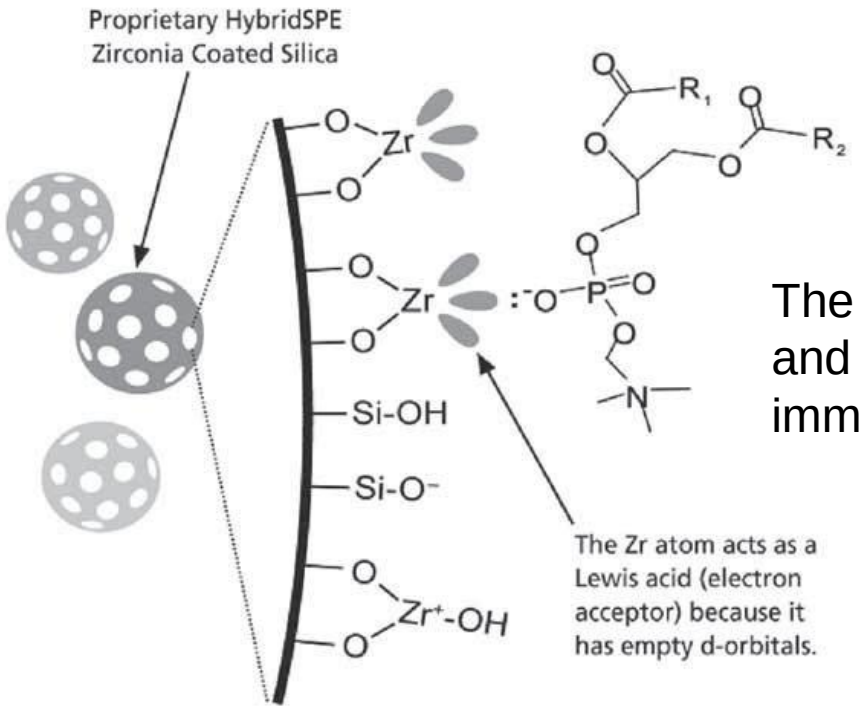
- cleanest extracts (removal of proteins, salts, and lipids)
- method development flexibility

SPE and LLE disadvantages:

- More difficult to automate and require time consuming optimization
- Final extract often not compatible with mobile phase
- Expensive and ineffective for high throughput
- Polar compounds can suffer from low recoveries
- Proteins and phospholipids can still co-elute
  - In both reversed phase SPE and LLE, the hydrophobic tail can result in co-extraction with analytes
  - In ion exchange and mixed mode SPE, the zwitterionic polar head group can result in co-extraction with basic or acidic analytes of interest

# HybridSPE-PPT®

The phospholipid retention mechanism on the HybridSPE-PPT is based on a highly selective Lewis acid-base interaction between the proprietary zirconia ions functionally bonded to the stationary phase and the phosphate moiety of phospholipids.




The resulting eluent is depleted of proteins and phospholipids and is ready for immediate LC-MS or LC-MS/MS analysis

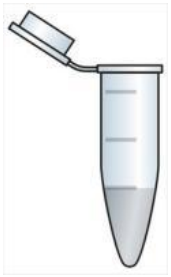
*Selective Depletion of Phospholipids in Bioanalysis using HybridSPE-PPT Technology – Chromatography Today – June 2009*

# Hybrid SPE-PPT vs PPT

- Selectivity evaluation of Hybrid SPE-PPT and PPT
- Three Merck proprietary compounds
  - with acidic (A), zwitterionic (B) and basic characteristics (C)
- Two plasma matrices
  - rat and human plasma
- Recovery evalution
- Matrix effect quantitation

# Experimental

## PPT



100 µL of plasma

300 µL $CH_3CN$ + 1% HCOOH

↓

Centrifugation

↓

Injection of Supernatant

## Hybrid SPE-PPT

100 µL of plasma

300 µL $CH_3CN$ + 1% HCOOH

↓

Filtration

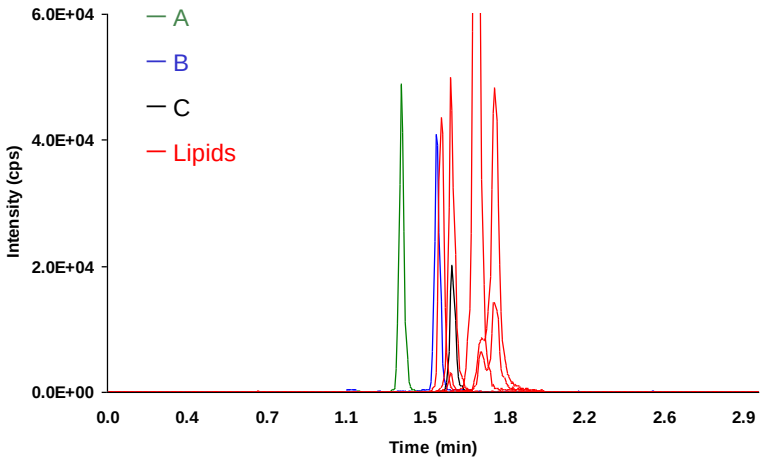
↓

Injection of Filtrate

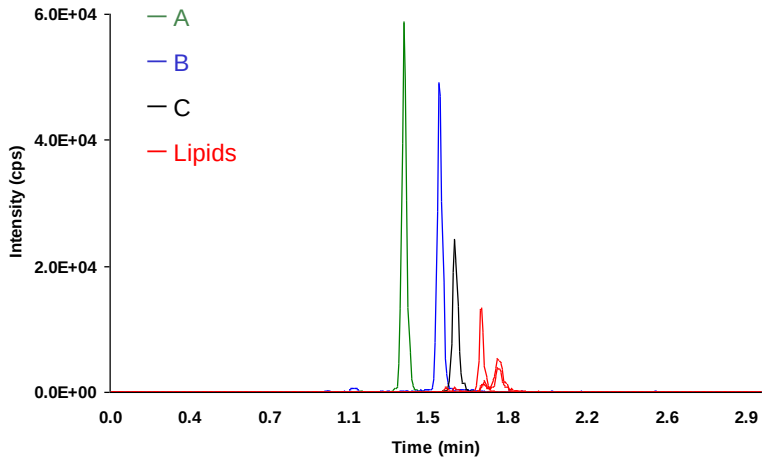
*J. Pharm. Biomed. Anal., 50, 867-871, (2009)*

# Hybrid SPE-PPT vs PPT – rat plasma

Rat Plasma after PPT




Rat Plasma after HybridSPE-PPT

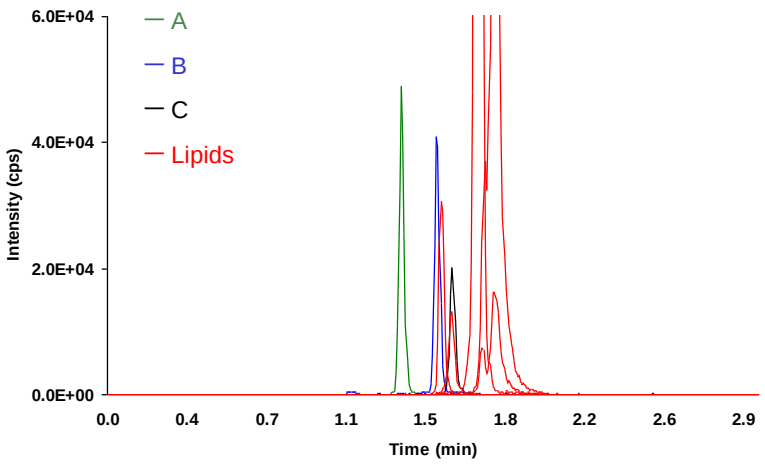



- Efficient removal of phospholipids with HybridSPE-PPT
- Reduction of phospholipid-based matrix effect for for all three model compounds

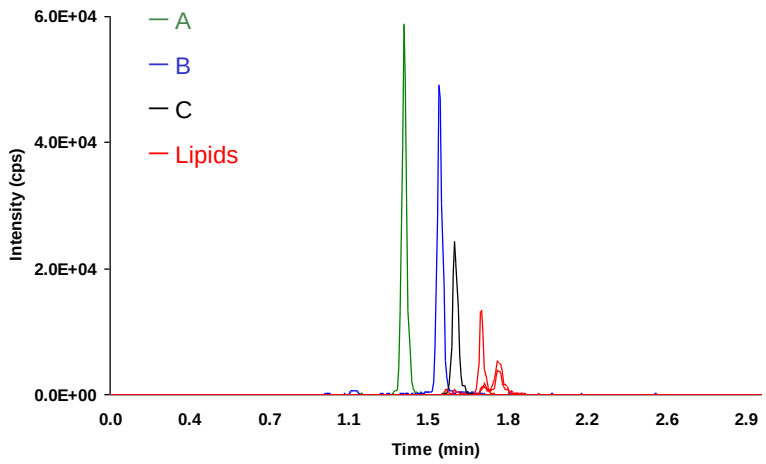
*J. Pharm. Biomed. Anal., 50, 867-871, (2009)*

# Hybrid SPE-PPT vs PPT – human plasma

Human Plasma after PPT



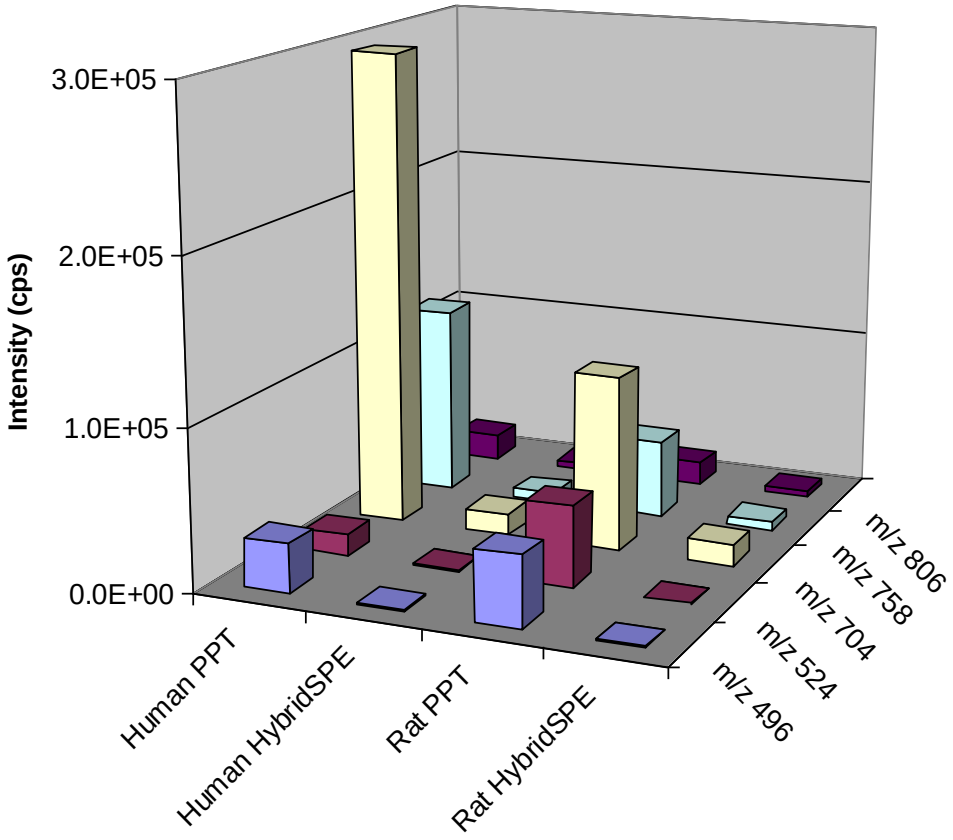

Human Plasma after HybridSPE-PPT




- Efficient removal of phospholipids with HybridSPE-PPT
- Significant reduction of phospholipid-based matrix effect for all three model compounds

*J. Pharm. Biomed. Anal., 50, 867-871, (2009)*

# Phospholipids depletion efficiency

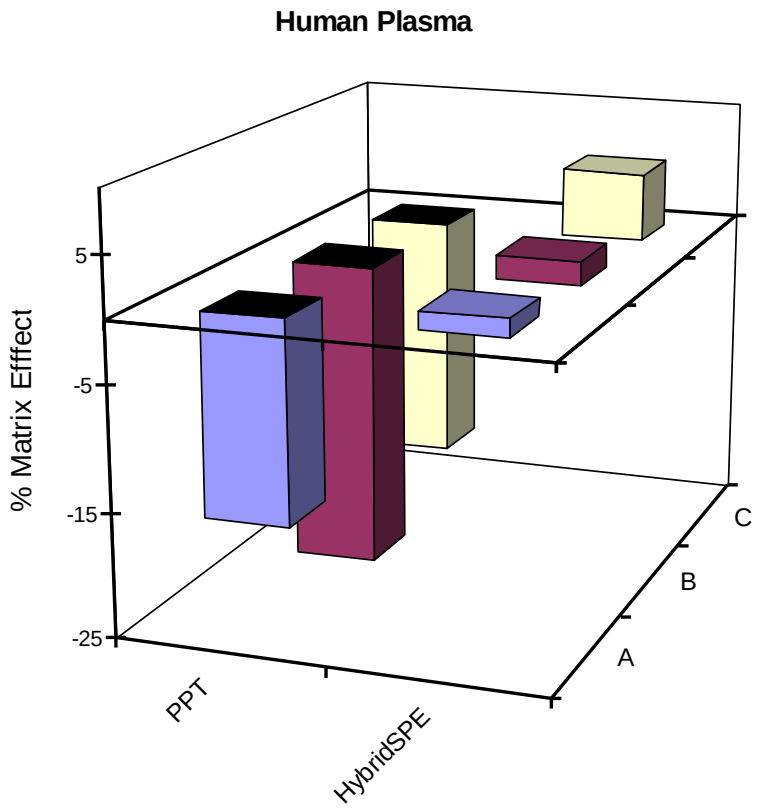


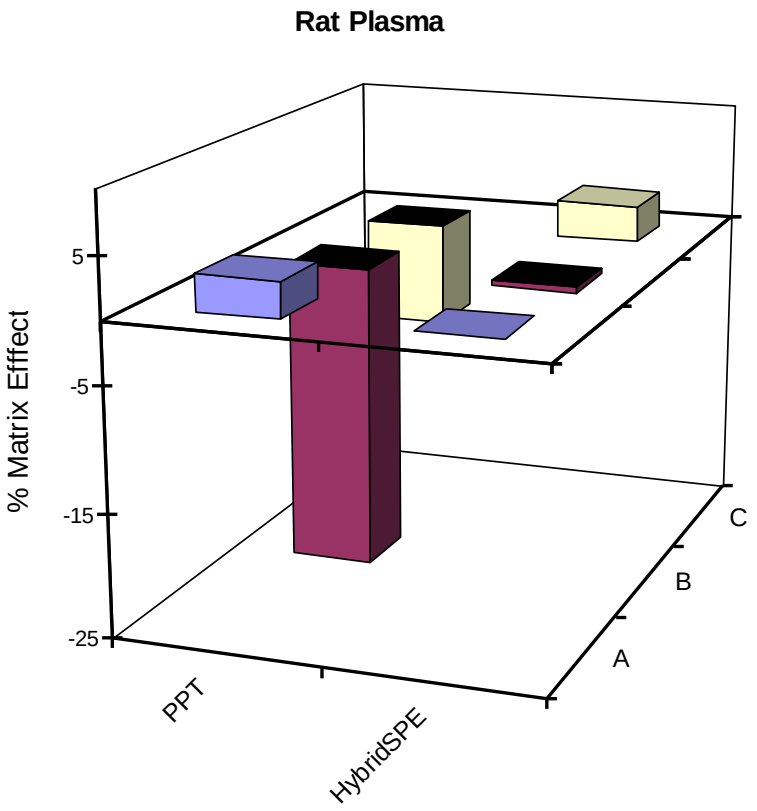

| % Lipids Removal | | | | | |
|---|---|---|---|---|---|
| | m/z 496 | m/z 524 | m/z 704 | m/z 758 | m/z 806 |
| Human | 97.6 | 93.5 | 95.5 | 95.5 | 76.5 |
| Rat | 98.3 | 98.3 | 87.7 | 89.0 | 73.0 |

*J. Pharm. Biomed. Anal., 50, 867-871, (2009)*

# Matrix effect evaluation

The matrix effect is significantly reduced when the HybridSPE-PPT sample clean-up procedure is used

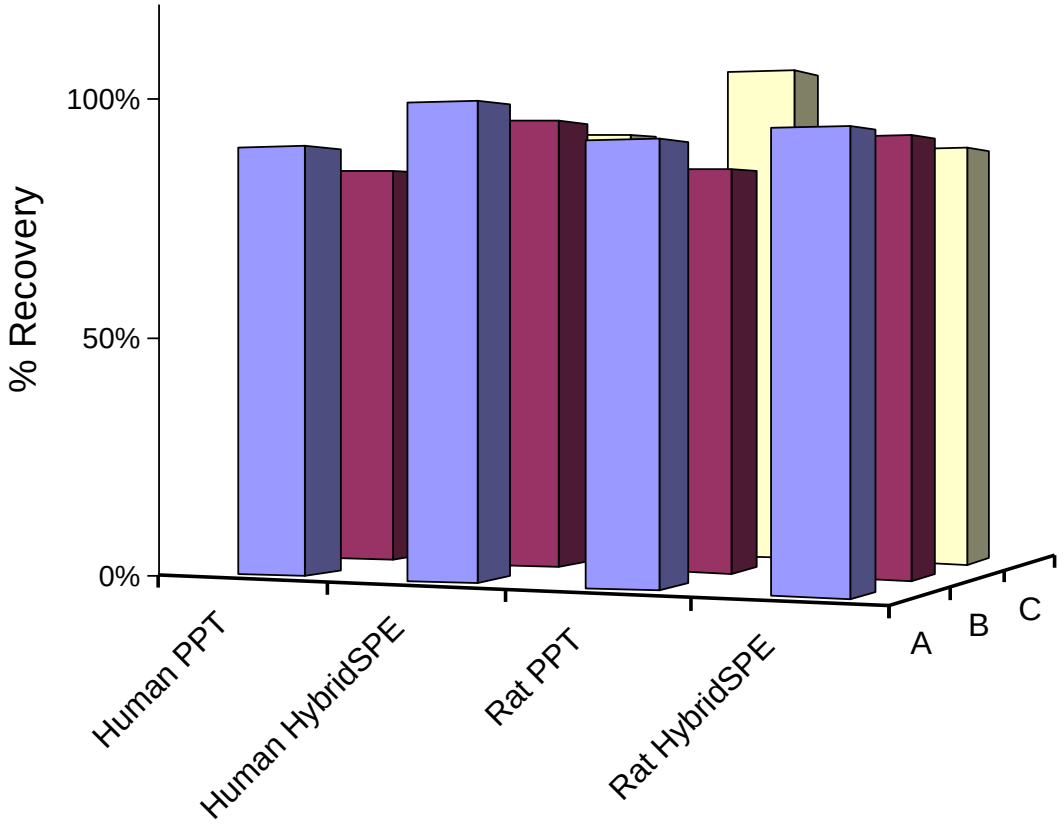



*J. Pharm. Biomed. Anal., 50, 867-871, (2009)*

# Extraction yield evaluation

The extraction yield was determined as the response of a post spiked blank sample extracts versus the same extracted sample. Recovery values ranged from 73.9 to 124.9% confirming the high efficiency of the HybridSPE-PPT




*J. Pharm. Biomed. Anal., 50, 867-871, (2009)*

# Conclusion

PPT can sometimes fulfill the requirements of extraction yield, but is not adequate to sufficiently remove phospholipids

LLE extraction followed by extraction on cerium columns, or PPT with colloidal silica plasma pretreatment as well as SPE in mixed-mode and supported liquid extraction selectively remove plasma phospholipids

The phospholipids MRM transitions monitoring and the matrix effect measurements clearly indicated that endogenous phospholipids particularly in human plasma PPT extracts are cause of ion suppression for the analytes of interest

HybridSPE-PPT is a useful and effective tool in providing cleaner extract and reducing or eliminating phospholipids-based matrix effects

# Acknowledgements

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Kevin Bateman
Eric Woolf
Roger Hayes

*Richard Abbott*

# Back-up

# % Matrix Effect = (A - B)/B x 100

Where, B is the peak area of a neat standard and A is the corresponding peak area for standards spiked into plasma after extraction.

| Analyte | Concentration ng/mL | PPT | | HybridSPE-PPT | |
|---|---|---|---|---|---|
| | | Rat | Human | Rat | Human |
| A m/z 474.30 | 5 | 11.5 ±13.3 | -14.0 ± 8.5 | 10.0 ± 1.5 | -0.5 ± 1.6 |
| | 50 | 7.8 ± 4.1 | -14.5 ± 2.4 | -1.9 ± 4.3 | 1.5 ± 1.4 |
| | 500 | 2.9 ± 3.0 | -16.4 ± 3.4 | 0.1 ± 6.1 | 1.5 ± 4.0 |
| B m/z 461.30 | 5 | -24.9 ± 2.5 | -34.8 ± 2.6 | -4.9 ± 4.7 | -5.1 ± 3.0 |
| | 50 | -32.2 ± 2.0 | -32.0 ± 1.1 | -10.4 ± 1.5 | -8.4 ± 0.2 |
| | 500 | -28.1 ± 0.7 | -24.9 ± 0.6 | -0.5 ± 5.0 | 1.9 ± 2.4 |
| C m/z 766.50 | 5 | 5.0 ± 7.6 | -25.5 ± 12.1 | 5.0 ± 0.9 | 15.0 ± 5.3 |
| | 50 | -5.0 ± 4.5 | -21.2 ± 2.8 | 6.0 ± 2.0 | 3.5 ± 4.5 |
| | 500 | -8.6 ± 1.0 | -20.3± 2.6 | 3.0 ± 1.6 | 5.6 ± 4.8 |

# % Recovery = (C / A) x 100 %

Where, *C* is the response for the extracted samples and *A* the response for post-extracted spiked samples.

| Analyte | Concentration ng/mL | PPT | | HybridSPE-PPT | |
|---|---|---|---|---|---|
| | | Rat | Human | Rat | Human |
| A m/z 474.30 | 5 | 113.5 ± 0.9 | 126.6 ± 11.5 | 103.8 ± 5.6 | 124.9 ± 2.0 |
| | 50 | 119.8 ± 3.4 | 112.2 ± 4.0 | 98.6 ± 2.6 | 111.2 ± 3.4 |
| | 500 | 90.2 ± 2.4 | 98.7 ± 2.8 | 91.8 ± 1.2 | 90.0 ± 4.5 |
| B m/z 461.30 | 5 | 99.7 ± 2.5 | 125.3 ± 6.9 | 109.9 ± 21.1 | 87.2 ± 11.7 |
| | 50 | 98.3 ± 2.3 | 112.2 ± 1.4 | 117.1 ± 12.5 | 73.9 ± 13.7 |
| | 500 | 92.0 ± 2.3 | 96.8 ± 1.2 | 85.6 ± 1.2 | 86.5 ± 2.5 |
| C m/z 766.50 | 5 | 86.2 ± 27.4 | 132.9 ± 13.1 | 109.5 ± 5.3 | 81.6 ± 7.3 |
| | 50 | 97.3 ± 4.8 | 108.2 ± 2.4 | 113.6 ± 5.0 | 116.6 ± 2.9 |
| | 500 | 91.1 ± 3.0 | 95.5 ± 4.0 | 108.9 ± 3.5 | 94.0 ± 4.6 |