A NOVEL SAMPLE PREPARATION DEVICE FOR IMPROVED PHOSPHOLIPID REMOVAL IN BIOANALYTICAL ASSAYS

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ABSTRACT

A new sample preparation device, the Ostro™ 96-well plate, aimed at the improved removal of phospholipids was used to develop bioanalytical methods for the routine analysis of several different analyte types. Performance of this sample prep device was compared to previously existing phospholipid removal products, protein precipitation (PPT), liquid-liquid extraction (LLE) and solid supported liquid-liquid extraction (SSLE) on the basis of lipid removal, reproducibility, analyte recovery and ease of use without further method development. Using the Ostro™ 96-well plate, a simple pass through clean-up provides high analyte recoveries for a broad spectrum of acidic, basic and neutral compounds while removing more endogenous phospholipids than similar devices. Reproducibility of phospholipid removal is significantly better than pre-existing lipid removal devices. In addition to characterization experiments, performance of this plate is demonstrated with a bioanalytical method for risperidone, 9-OH risperidone and its internal standard (IS) clozapine. Standard curves are linear over 4 orders of magnitude and QC sample accuracy and precision is better than 15%. Method simplicity, reproducibility, and highly efficient phospholipid removal make this technology an attractive alternative to LLE or PPT for obtaining higher quality data from large numbers of samples. In addition, the generic nature of this device and the significant clean-up provided facilitate productivity increases by reducing instrument downtime.

INTRODUCTION

The Ostro™ 96-well plate provide a novel solution for the cleanup of phospholipids in biological samples prior to LC/MS/MS analysis. Using a convenient 96-well format, in-well protein precipitation is performed using a single pass through method which provides consistent, high quality results: removal of significantly more phospholipids for cleaner extracts, optimal recovery for diverse analytes, and increased reproducibility for consistent, robust methods.

Ostro™
SAMPLE PREPARATION PRODUCTS

EXPERIMENTAL

System: Waters ACQUITY UPLC® System with a Waters Quattro Premier XE™ or Xevo™ TQ MS triple quadrupole mass spectrometer operated in positive ion MRM mode

Column: ACQUITY UPLC® HSS T3 2.1 × 150 m (used for the characterization experiments), ACQUITY UPLC® BEH C_{18} 2.1 × 50 mm, 1.7 µm (used for the risperidone application)

Mobile Phases A: 10mM ammonium formate in H_2O (pH 3)
B: MeOH

Gradient for characterization experiments:
1% B to 99% B in 2 min (0.5 min hold at 98% B before return to initial conditions) or 1% B to 99% B in 7 min, hold at 99% B until 12 min, return to initial conditions

Gradient for risperidone application:
25% B to 75% B in 3 min (1 min hold at 98% B before return to initial conditions)

Flow Rate: 0.6 mL/min
Injection: 8 µL
Temperature: 35 °C
Desolvation Gas Flow: 800 L/hr
Source Temperature: 130 °C

METHODS

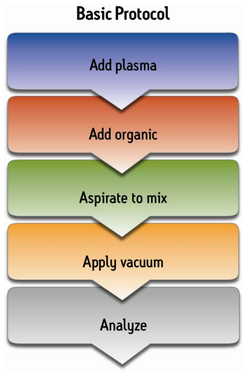

Figure 1: Basic protocol for Ostro™ 96-well Plates

RESULTS

Level of Phospholipids Remaining

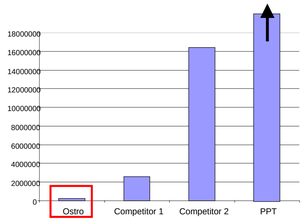

Figure 2: Comparison of phospholipids remaining in extracted samples using Ostro™ 96-well plate, two competitive phospholipid removal plates, and PPT.

Level of Phospholipids Remaining

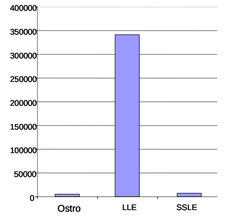

Figure 3: Comparison of phospholipids remaining in extracted samples prepared using the Ostro™ 96-well plate, LLE and SSLE.

Reproducibility of Phospholipid Removal

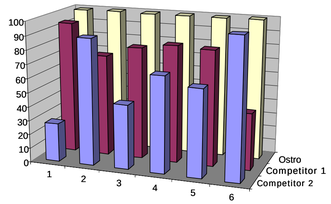

Figure 4: % phospholipid removal in 6 lots of human plasma, comparing the Ostro™ 96-well plate to 2 competitive phospholipid removal plates

Average Analyte Recovery

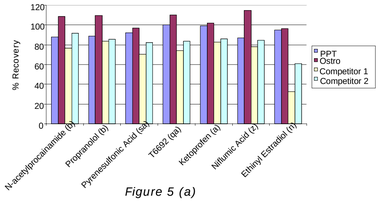

Figure 5 (a)

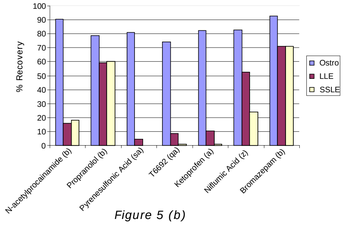

Figure 5 (b)

Figure 5 (a): Average analyte recovery of a diverse analyte mix using protein precipitation, Ostro™ 96-well plate, 2 competitive phospholipid removal 96-well plates and (b) LLE and SSLE.

BIOANALYTICAL METHOD FOR RISPERIDONE AND METABOLITE: ANALYTICAL CHALLENGES

- Broad linear dynamic range: 0.025-500 ng/mL
- Must meet accuracy and precision requirements for standards and QC samples and all regulatory requirements
- Reproducible
- Meet detection limit need
- Easy
- Fast
- No method development

SENSITIVITY AND LINEARITY

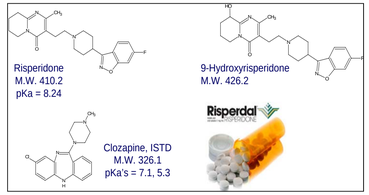

Figure 6. Structures and chemical properties of Risperidone, 9-hydroxyrisperidone, and the internal standard, Clozapine.

QC (ng/mL)	Average Calculated Concentration (ng/mL)	%RSD
0.075	0.083	11.3
0.75	0.762	1.5
7.5	7.62	1.6
75	75.932	1.2

Table 1: Representative quality control sample calculations for Risperidone

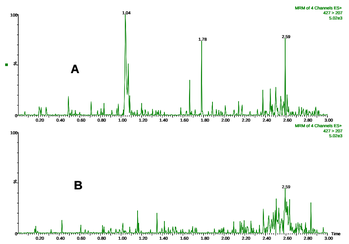

Figure 7: UPLC®/MS/MS representative chromatogram of the XIC for a sample of 9-OH risperidone in rat plasma at the LLOQ of 0.025 ng/mL (A), and extracted blank plasma (B).

DISCUSSION

Using a simple protocol *(Figure 1)* and a set of diverse analytes, Ostro™ 96-well plate was directly compared to PPT, 2 competitive phospholipid removal 96-well plates, LLE, and SSLE on the basis of phospholipid removal, reproducibility of phospholipid removal, and analyte recovery. In terms of phospholipid removal, Ostro™ reproducibly removes significantly more phospholipids than the current phospholipid removal products on the market, PPT, LLE, and SSLE *(Figures 2, 3, and 4)*.The Ostro™ 96-well plate provided the highest overall recovery for all analytes tested in the diverse mix (*Figure 5*). The average RSD for lipid removal across six sources of human plasma using Ostro™ was <1%, while competitive lipid removal plates exhibited RSD's between 24 and 41% for lipid removal. The generic nature of this device and the significant clean-up provided facilitate productivity increases by reducing systems downtime. Finally, a practical application for risperidone and its hydroxylated metabolite (*Figure 6)* was performed using the Ostro™ 96-well plate. Calibration curves were linear over greater than 4.5 orders of magnitude and points on the curve and quality control samples easily met accuracy and precision requirements (*Table 1.*) Representative statistics for the risperidone standard curve were as follows:
Correlation coefficient r = 0.997938, r^2 = 0.995879
Weighting: 1/x^2
The required LLOQ of 0.025 ng/mL in rat plasma (*Figure 7)* was easily achieved.

CONCLUSION

Providing cleaner, more reproducible extracts than competitive phospholipid removal devices or other techniques, Ostro™ 96-well plates allow for more sensitive analyses, significantly cleaner samples, and increased sample throughput. Requiring minimal to no method development, this technology can be rapidly implemented to improve laboratory workflow and increase instrument uptime.

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