The Utility of Magnetic Beads as an Extraction Technique for Small Molecule Bioanalysis by LC-MS/MS

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Background

Automation

- Bioanalysis looking to automate various processes including protein precipitation (PPT)
- Long term goal of end to end automation
- Centrifugation is difficult to automate
  - Movement of samples and plates
  - Balancing of tubes
- Magnetism established in Biopharm workflows
  - Fast
  - Effective
  - Automated
- Looked to Clinical Chemistry for ideas
  - Evaluate MagSiMUS-TDM$^\text{PREP®}$ beads
Background

Beads Vs PPT

- What advantages do beads offer over PPT
  - Fast ✓
  - Easy ✓
  - Cheap ×
  - Efficiency/Effectiveness of extraction ?

- Movement of samples
- Balancing plates

- Automation
  - Kingfisher
  - MagSiMUS\textsuperscript{DX}

- No direct cost savings

- Seeking to establish
The Beads Tested

- **MagSiMUS-TDM\textsuperscript{PREP®}** magnetic beads from MagnaMedics
  - Negative Selection for removal of proteins and other impurities
  - Contaminants (plasma proteins and large peptides) precipitated onto the beads surface
  - Bead attracted to the magnet
  - Analytes of interest remain in solution ready for direct injection on LC-MS
- 2 bead types available (TI and TII)
- Combined with an acetonitrile based (OPR I) or a methanol based (OPR VI) organic precipitation reagent.
Bead Extraction Protocol

- 50 µL Matrix
- 20 µL ISTD Solution
- 40 µL Type I or Type II beads
- 200/250 µL of Organic Precipitation Reagent (OPR) I or VI, mix
- Sample then placed on a magnetic separator for 2 minutes
- Supernatant transferred to clean tube
- Whole blood requires a lysis step
Efficiency of Beads Vs PPT

- Residual protein content
- Removal of Phospholipids
- Recovery of analyte of interest
- Matrix effects
Residual Protein content

- Absorbance at 280nm
- Plasma from 3 species
- Multiple plasma batches
- TI, TII plus a mix of the two compared to PPT
- Mean percentage protein decrease calculated
Residual Protein Content

Mean Percentage Protein Decrease in Rat, Dog and Human Plasma

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Dog</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>TI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI&amp;TII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean protein decrease (%)</td>
<td>93</td>
<td>97</td>
<td>96</td>
</tr>
</tbody>
</table>

95  94  93  92  91  90  98  97  96  95  94  93  92  91  90
Almost 70% of plasma phospholipids are Phosphatidylcholines

- PPT does not remove these
- Generally late eluting can cause interference in later injections
- 5 MS/MS transitions monitored

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Parent Ion Mass</th>
<th>Product Ion Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-palmitoyl-2-hydroxy-sn-glycerol-3-phosphocholine</td>
<td>496.35</td>
<td>184.3</td>
</tr>
<tr>
<td>1-stearoyl-2-hydroxy-sn-glycerol-3-phosphocholine</td>
<td>524.37</td>
<td>184.3</td>
</tr>
<tr>
<td>1-hexade-canyol-2-(9Z, 12Z-octadecadienoyl)-sn--glycerol-3-phosphocholine</td>
<td>758.57</td>
<td>184.3</td>
</tr>
<tr>
<td>1-(9Z, 12Z-octadecadienoyl)-2-5Z, 8Z, 11Z14Z-eicosatetraenoyl)-sn--glycerol-3-phosphocholine</td>
<td>806.57</td>
<td>184.3</td>
</tr>
<tr>
<td>Glycerophosphocholine lipid</td>
<td>703.57</td>
<td>184.3</td>
</tr>
</tbody>
</table>
Phospholipid Content

Total phospholipid response

- Rat
  - TI
  - TII
  - TI+TII
  - PPT

- Dog
  - TI
  - TII
  - TI+TII
  - PPT

- Human
  - TI
  - TII
  - TI+TII
  - PPT

Total phospholipid in each sample after preparation with PPT, TI and TII beads.
Mean Percentage Recovery Across 3 Compounds in Rat Plasma

<table>
<thead>
<tr>
<th>Compound</th>
<th>TI</th>
<th>TII</th>
<th>PPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>73.99</td>
<td>67.33</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>82.99</td>
<td>73.98</td>
<td>54.83</td>
</tr>
<tr>
<td>C</td>
<td>80.46</td>
<td>87.97</td>
<td>84.07</td>
</tr>
</tbody>
</table>
Recovery

- Compound A (mw 358, logP 3.40) showed the poorest recovery with the beads, with approximately 9% better recovery with PPT.
- Compound B (mw 458, logP 3.86) Both type I and II beads giving markedly better recovery than PPT with type I beads giving in the region of 30% more recovery.
- Compound C (mw 410, logP 2.12) Gave fairly equal recovery across the board, type II beads marginally having marginally the greatest recovery.
Matrix Effects

- Mean data from 3 compounds for each species
- Beads performing least well in human plasma, both in terms of across species and against PPT
- Type I beads showing the least matrix effect in both rat and dog plasma
Conclusions

- Beads showed an advantage over PPT in
  - Removal of phospholipids
  - Recovery (2 from 3)
  - Matrix effects (at least one bead type in rat and dog plasma)
  - Simple to automate (no centrifugation step) with hybrid workflows automation already established in labs

- Overall the MagSiMUS-TDM<sup>PREP®</sup> beads show promise as a sample preparation technique, more characterisation is needed
  - More compounds
  - Experimental conditions
  - Batch to batch variability and robustness
  - Potential for new bead types optimised for bioanalysis in collaboration with the manufacturers
Acknowledgements

- Ella Boardman
- Adam Hughes
- Teresa Heslop
- Scott Summerfield
- Peter van Driessche, MagnMedics
Matrix Effects

- Matrix factor was calculated

- Suppression was calculated from MF and mean was taken

\[ Supression = 1 - MF \]
Glycerophosphocholine lipid

Sample type

Response

RTPL TI  RTPL TII  RTPL TI+TII

RTPL PPT  DOPL TI  DOPL TII

DOPL TI+TII  DOPL PPT  HUPL TI

HUPL TII  HUPL TI+TII  HUPL PPT
1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphocholine
1-palmitoyl-3-hydroxy-sn-glycero-3-phosphocholine
1-stearoyl-2-hydroxy-sn-glycero-3-phosphocholine

Response

Sample type

RTPL TI  | RTPL TII | RTPL TI+TII | RTPL PPT | DOPL TI  | DOPL TII | DOPL TI+TII | DOPL PPT  | HUPL TI  | HUPL TII | HUPL TI+TII | HUPL PPT

1
- stearoyl
2
- hydroxy
3
- sn-glycero
4
- phosphocholine
1-(9Z,12Z-octadecadienoyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine