An Ultrasonic Assay for Quantification of a Cationic Lipid, 1,2-Dioleoyl-3-Trimethylammonium Propane, in Mouse Plasma by HPLC-MS/MS
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Introduction
Lipids are widely used as carriers for delivery of therapeutic molecules by forming liposomes or nanoparticles. 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP) (Figure 1) is a synthetic cationic lipid that is widely used in liposomal and nanoparticle drug carrier systems. Due to the increasing use of DOTAP liposomes for the encapsulation and targeting of cytostatic drugs, it is now necessary to accurately quantify the lipids in the biological material. A previous publication described an assay to quantify DOTAP in human plasma; however, the assay had a time-consuming extraction and lacked sufficient sensitivity (LLOQ=10 ng/mL). Here we report an ultrasonic, fast, selective and robust assay for the quantification of DOTAP in mouse plasma by HPLC-MS/MS with a wide range of 0.05-50.0 ng/mL.

Methodology
Sample Preparation
The samples containing analyte and internal standard were extracted from 25.0 µL of mouse plasma using protein precipitation extraction. The extracts are stored at 5°C while waiting for analysis.

Chromatographic Conditions
Column: XSELECT CSH C18 (3.5µm, 30 x 2.1 mm)
Mobile Phase: A: MeOH and MeCN
Injection volume: 10 µL
Column temperature: 45°C
Optimization of LC Conditions and IS Selection
Isopropanol was used as a mobile phase component to achieve optimum chromatographic peak shape for both the analyte and the IS. In order to eliminate injector carryover, needle washes composed of IPA, methanol, MeCN, formic acid and water were used. Different analog internal standards were screened, and DOTMA demonstrated the best ability to track the analyte during extraction and MS analysis. No contribution was observed between DOTAP and DOTMA (Figure 3). Different fragments for DOTAP (including m/z 603.5, 380.3 and 265.2), and DOTMA (including m/z 97.1, 83.0 and 69.1) were evaluated, and m/z 603.5 (DOTAP) and m/z 69.1 (DOTMA) provided not only best sensitivity, but also the cleanest background.

Results
Method Development
Amoeba Sample Extraction Protocol™ (Covance) was applied for the optimization of the extraction procedure. In the Amoeba™ protocol, different extraction technologies including SPE, PPE, SLE and LLE, were evaluated. The Protein precipitation methodology provided the best recovery (Figure 2). In order to pursue a lower LOQ and improve the overall recovery, the parameters of the PPE procedure and all PPE solvents (e.g. composition, pH, volume, etc.) were further optimized. A basic buffer along with IPA/MeOH provided both improved recovery and precision for the extraction.

Conclusions
This assay for the quantification of DOTAP in mouse plasma is an ultrasonic, selective and robust LC-MS/MS assay, and is the most sensitive LC-MS assay published thus far for the determination of DOTAP in mouse plasma.

References

Table 1. Back-Calculated Concentrations of Calibration Standards for DOTAP
<table>
<thead>
<tr>
<th>Compound</th>
<th>Nominal Conc.</th>
<th>LOQ QC</th>
<th>Low QC</th>
<th>Low-Medium QC</th>
<th>Medium QC</th>
<th>High QC</th>
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</thead>
<tbody>
<tr>
<td>DOTAP (Analyte)</td>
<td>0.050 ng/mL</td>
<td>0.100 ng/mL</td>
<td>0.500 ng/mL</td>
<td>2.500 ng/mL</td>
<td>10.0 ng/mL</td>
<td>50.0 ng/mL</td>
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<td></td>
<td>Mean Observed Conc.</td>
<td>0.0498</td>
<td>0.101</td>
<td>0.500</td>
<td>2.39</td>
<td>10.2</td>
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<td>%Bias</td>
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<td>-0.100</td>
<td>-4.39</td>
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<td>Total Variation (%CV)</td>
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<td>5.57</td>
<td>2.60</td>
<td>5.73</td>
<td>6.82</td>
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<tr>
<td>IS 634.6</td>
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<td>0.100 ng/mL</td>
<td>0.500 ng/mL</td>
<td>2.500 ng/mL</td>
<td>10.0 ng/mL</td>
<td>50.0 ng/mL</td>
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<td>Mean Observed Conc.</td>
<td>0.050</td>
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<td>0.500</td>
<td>2.39</td>
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<td>0.96</td>
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<td>4.6</td>
<td>6.4</td>
<td>2.8</td>
<td>5.7</td>
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</tbody>
</table>

Table 2. Intra-Assay Accuracy and Precision Quality Control Samples for DOTAP

Figure 1. The structure of DOTAP.

Figure 2. Amoeba extraction screening data for DOTAP in mouse plasma.

Figure 3. Chromatograms of analyte only and IS only.

Figure 4. DOTAP chromatograms of LLOQ (0.050 ng/mL).