In situations where high levels of endogenous analyte are present, a surrogate matrix is often used. Our strategy, however, is to utilize a stable isotope (d5) labeled form of cholesterol as a surrogate analyte as well as a modified surrogate matrix. As cholesterol-d5 is practically insoluble in aqueous solutions (<5 µg/mL), the preparation of calibration standards and QC samples to cover a standard curve range of 500 to 5000 µg/mL is therefore performed after a 50-fold dilution of human plasma in phosphate buffered saline: 2-propanol (90:10).

Results

The calibration curves are linear in the range of 500 to 5000 µg/mL, using a 1/8 weighted regression.

Validation Data: Precision and Accuracy

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>LLQC QC 500</th>
<th>LC QC 900</th>
<th>HQC QC 1750</th>
<th>HQC QC 3850</th>
<th>HQC QC 14600</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>497</td>
<td>919</td>
<td>1780</td>
<td>3930</td>
<td>1460</td>
</tr>
<tr>
<td>Inter-run RSD (%)</td>
<td>10.9</td>
<td>5.5</td>
<td>6.0</td>
<td>6.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Inter-run %Bias</td>
<td>-6.6</td>
<td>-2.1</td>
<td>-0.6</td>
<td>-2.1</td>
<td>-1.4</td>
</tr>
</tbody>
</table>

Stability

Established Stability of Cholesterol and Cholesterol-d5 Plasma Diluted 50 fold in PBS: 2-propanol (90:10)

- Frozen at -20°C for 134 days
- Frozen at -80°C for 134 days
- 4 freeze-thaw cycles
- 24 hours storage at room temperature

Extracted/Processed Samples

- 143 hours at 5°C

Established Stability of Endogenous Total Cholesterol Undiluted Plasma

- Frozen at -20°C for 106 days
- Frozen at -80°C for 106 days
- 4 freeze-thaw cycles
- 24 hours storage at room temperature

Whole Blood

- 2 hours at room temperature or on wet ice

Conclusions

- There is increasing interest in the use of plasma total cholesterol levels for normalization of plasma concentrations of the biomarker 4β-hydroxycholesterol.
- The use of a surrogate analyte is a useful tool to circumvent endogenous interference in the assay of plasma total cholesterol.
- Covance has validated a robust method for the determination of total cholesterol in human plasma. This method has been used successfully in the analysis of unknown samples in support of the evaluation of the biomarker 4β-hydroxycholesterol.