Introduction

Choline concentrations in complex matrices can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI’s unique enzyme technology provides for specific choline measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a sample is injected into the sample chamber, the choline diffuses into the membrane containing choline oxidase. The choline is immediately oxidized to hydrogen peroxide and betaine. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, and hence to choline concentration.
I. Materials & Setup

A. YSI Series Biochemistry Analyzer - equipped with a 2771 Choline Membrane and 2357 Buffer.
B. Choline standards (175 mg/L, 450 mg/L).
C. Connect the YSI Series Biochemistry Analyzer to a suitable power source.
D. Perform the instrument and membrane daily checks described in the User’s Manual (Section 5).
E. Volumetric glassware (Class A recommended).
F. The following instrument setup is recommended.
   Sample size: 25 μL
   \[
   \begin{array}{|c|c|}
   \hline
   \text{Probe A Parameters} & \text{Autocal Parameters} \\
   \text{Chemistry} & \text{Choline} \\
   \text{Unit} & \text{mg/L (ppm)} \\
   \text{Calibrator} & 175 \text{ mg/L} \\
   \text{End Point} & 30 \text{ Sec} \\
   \hline
   \text{Temperature} & 1 ^\circ \text{C} \\
   \text{Time} & 30 \text{ Min} \\
   \text{Sample} & 2 \text{ Sample} \\
   \text{Cal Shift} & 2\% \\
   \hline
   \end{array}
   \]
   \[
   \begin{array}{|c|c|}
   \hline
   \text{Probe B Parameters} & \text{Autocal Parameters} \\
   \text{Chemistry} & \text{None} \\
   \text{Temperature} & 1 ^\circ \text{C} \\
   \text{Time} & 30 \text{ Min} \\
   \text{Sample} & 5 \text{ Samples} \\
   \text{Cal Shift} & 1\% \\
   \hline
   \end{array}
   \]
II. Method

A. Total choline concentration should not exceed 450 mg/L, as determined on Part D below; otherwise the sample will require further dilution. Use volumetric glassware for all dilutions. Dilute with either water or 2357 buffer.
B. Calibrate the YSI Series Biochemistry Analyzer with a 175 mg/L Calibration Standard.
C. Check the linearity of the membrane at least once a day by injection of a choline linearity check solution (450 mg/L). Refer to the User’s Manual (Section 5) for specifications.
D. Assay the sample by aspiration into the YSI Series Biochemistry Analyzer. The linear range of the system is 5 to 450 mg/L choline. If the value reported exceeds this, further dilution is required.
E. Calibrate frequently as described in the User’s Manual (Section 7).

III. Calculations

To calculate % choline, multiply the reported value by the appropriate dilution factor.

Example: 5.0 grams of pet food and 100 mL of water were mixed in a blender for 5 minutes. The supernatant was analyzed for choline. The value reported was 77.0 mg/L choline.

\[
\frac{77.0 \text{ mg/L} \times 0.100}{5000 \text{ mg}} = 0.0015 \text{ g choline/g pet food}
\]

\[
= 0.15\% (w/w)
\]

Example: Infant formula was aspirated into the YSI Series Biochemistry Analyzer (no dilution). The choline content was as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg/L Choline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant Formula A</td>
<td>232</td>
</tr>
<tr>
<td>Infant Formula B</td>
<td>138</td>
</tr>
<tr>
<td>Medical Nutritional Formula A</td>
<td>398</td>
</tr>
<tr>
<td>Medical Nutritional Formula B</td>
<td>386</td>
</tr>
</tbody>
</table>
YSI Life Sciences develops and manufactures scientific instruments, sensors and systems that serve a variety of scientific and industrial markets worldwide. YSI has a long history in the life sciences and bioanalytical markets, most notably with our introduction of the world's first commercial whole blood glucose analyzer in 1975. Today there are over 10,000 YSI instruments installed around the world, trusted in critical situations to provide the most accurate data in the shortest time.

Ordering Information

YSI Part Numbers:
2900  Series Biochemistry Analyzer
2771  Choline Membrane Kit
2772  Choline Standard Solution (175 mg/L)
2773  Choline Standard Solution (450 mg/L)
2357  Buffer Kit
2363  Potassium Ferrocyanide Test Solution
2392  NaCl Solution (for membrane installation)