Estimation of Measurement Uncertainty:

Determination of Potency of Penicillin V Potassium by HPLC

Summary of Method

- **Prepare Standard Solution** - Accurately weigh 25 mg. of Pen. V Pot. RS of stated purity in to a 10 ml. volumetric flask, and dilute to volume with mobile phase.

- **Prepare Sample Solution** - Accurately weigh 125 mg. of Pen. V Pot. Sample in to a 50 ml. volumetric flask. Dilute to volume with mobile phase.

- **Measure Standard Solution** - Inject a volume of standard solution. Record peak area.

- **Measure Sample Solution** - Inject a volume of sample solution. Record peak area.

- **Determine potency of Pen. V Pot. in sample**

\[
\text{Potency (unit/mg)} = \frac{A_{\text{sam}} \times V_{\text{sam}} \times C_{\text{std}} \times P_{\text{std}}}{A_{\text{std}} \times M_{\text{sam}}} 
\]

where

- \( A_{\text{sam}} \) = Peak area for the sample solution
- \( A_{\text{std}} \) = Peak area of the standard solution
- \( C_{\text{std}} \) = Concentration of the standard solution (mg/ml)
- \( M_{\text{sam}} \) = Mass of the sample (mg)
- \( V_{\text{sam}} \) = Volume of sample solution (ml)
- \( P_{\text{std}} \) = Potency of Std = 1520 unit/mg
DATA

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
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<tr>
<td><strong>Tare+Load (g)</strong></td>
<td>0.038566</td>
<td>0.2413</td>
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<td><strong>Tare (g)</strong></td>
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<td><strong>Wt. Taken (g)</strong></td>
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<td>0.1255</td>
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<td>1924006.3</td>
<td>1918599.0</td>
<td>1950585.0</td>
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<td><strong>SD</strong></td>
<td>1552.9519</td>
<td>4255.7619</td>
<td>3106.119819</td>
<td>4369.1847</td>
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<tr>
<td><strong>%RSD</strong></td>
<td>0.08</td>
<td>0.22</td>
<td>0.16</td>
<td>0.23</td>
<td>0.26</td>
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<td><strong>Potency (unit/mg)</strong></td>
<td>1495.3116</td>
<td>1491.6695</td>
<td>1480.3658</td>
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<td><strong>SD</strong></td>
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<td><strong>%RSD</strong></td>
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</table>
Estimation of Measurement Uncertainty

1. Specify measurand

Measurand = Potency of Pen. V Pot. (unit/mg)

\[
Potency (unit/mg) = \frac{A_{\text{sam}} \times V_{\text{sam}} \times C_{\text{std}} \times P_{\text{std}}}{A_{\text{std}} \times M_{\text{sam}}}
\]

2. Identify Uncertainty sources

Cause and Effect diagram

- \(A_{\text{sam}}\)
- \(A_{\text{std}}\)
- \(C_{\text{std}}\)
- \(V_{\text{sam}}\)
- \(M_{\text{sam}}\)
- \(V_{\text{std}}\)
- \(M_{\text{std}}\)
- Rep.
- Calibration
- Temperature
- Repeatability
- (tare + gross)

Potency (unit/mg)
### Source of Uncertainty

<table>
<thead>
<tr>
<th>Source of Uncertainty</th>
<th>Quantify Procedure</th>
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</thead>
<tbody>
<tr>
<td>1. Uncertainty of A sam.</td>
<td>Repeatability of sample: Standard deviation of peak area of sample solution</td>
</tr>
<tr>
<td>2. Uncertainty of A std.</td>
<td>Repeatability of standard: Standard deviation of peak area of standard solution</td>
</tr>
</tbody>
</table>
| 4. Uncertainty of V sam. | - Tolerance of volumetric flask  
- Temperature Effect |
| 5. Uncertainty of C std. |  
5.1 Wt. of Std. | - Max. Uncertainty of Balance from Calibration Certificate |
| 5.2 Vol. of Std. Sol. | - Tolerance of volumetric flask  
- Temperature Effect |
| 6. Uncertainty of Method Precision | Repeatability of sample: Relative standard deviation of potency of sample |

### 3. Standard Uncertainty

1. **Peak area of Sample**
   
   Maximum standard deviation of peak area of sample solution  
   
   Standard deviation = 5017.543  
   
   **Standard Uncertainty of A sam,** $u(A_{sam}) = \pm 5017.543$

2. **Peak area of Standard**
   
   Standard deviation of peak area of standard solution  
   
   Standard deviation = 1552.9519  
   
   **Standard Uncertainty of A std,** $u(A_{std}) = \pm 1552.9519$

3. **Mass of Sample (tare + gross)**
   
   Max. Uncertainty of Balance from Calibration Certificate = ± 0.00039 g., $k = 2.25$  
   
   Standard Uncertainty = ($\pm 0.00039/2.25$) = ± 0.00017 g. = ± 0.17 mg.  
   
   Contributed by twice (tare + Gross)  
   
   $$u(M_{sam}) = \sqrt{0.17^2 + 0.17^2} = 0.2404$$  
   
   **Standard Uncertainty of M sam,** $u(M_{sam}) = \pm 0.24$ mg
4. Volume of Sample

- Calibration
  
  Tolerance of volumetric flask = ± 0.06 ml, 
  
  Standard uncertainty is calculated as a Rectangular distribution (divisor = \(\sqrt{3}\))
  
  Standard Uncertainty \( (u_{\text{CAL}}) = \pm 0.06/\sqrt{3} = \pm 0.0346 \) ml

- Temperature Effect
  
  The coefficient of volume expansion for water = \(2.1 \times 10^{-4}\) °C\(^{-1}\)
  
  Laboratory temperature varies between ±3 °C
  
  Volume variation = ±\((50 \times 3 \times 2.1 \times 10^{-4})\) = ± 0.0315 ml.
  
  Standard uncertainty is calculated as a rectangular distribution (divisor = \(\sqrt{3}\))
  
  Standard Uncertainty \( (u_{\text{TEMP}}) = \pm 0.0315/\sqrt{3} = \pm 0.0185 \) ml

Combining these contributions:

\[
 u(V_{\text{sam}}) = \sqrt{u_{\text{CAL}}^2 + u_{\text{TEMP}}^2} \\
 = \sqrt{0.0346^2 + 0.00185^2} \\
 = 0.0346
\]

**Standard uncertainty of V_{sam}, u(V_{sam}) = ± 0.0346 ml**

5. Concentration of Standard Solution

5.1 Volume of standard solution

- Calibration
  
  Tolerance of volumetric flask = ± 0.04 ml,
  
  Standard uncertainty is calculated as a Rectangular distribution (divisor = \(\sqrt{3}\))
  
  Standard Uncertainty \( (u_{\text{CAL}}) = \pm 0.04/\sqrt{3} = \pm 0.0231 \) ml

- Temperature Effect
  
  The coefficient of volume expansion for water = \(2.1 \times 10^{-4}\) °C\(^{-1}\)
  
  Laboratory temperature varies between ±3 °C
  
  Volume variation = ±\((10 \times 3 \times 2.1 \times 10^{-4})\) = ± 0.0063 ml.
  
  Standard uncertainty is calculated as a rectangular distribution (divisor = \(\sqrt{3}\))
  
  Standard Uncertainty \( (u_{\text{TEMP}}) = \pm 0.0063/\sqrt{3} = \pm 0.0036 \) ml
Combining these contributions:

\[ u_V = \sqrt{u_{CAL}^2 + u_{TEMP}^2} \]

\[ = \sqrt{0.0231^2 + 0.0036^2} \]

\[ = 0.0234 \]

Standard uncertainty of \( V \) std., \( u_V = \pm 0.0234 \) ml

5.2 Mass of Standard (tare + gross)

Max. Uncertainty of Balance from Calibration Certificate = \( \pm 0.026 \) mg., \( k = 2.05 \)

Standard Uncertainty = \( (\pm 0.026/2.05) = \pm 0.0127 \) mg.

Contributed by twice (tare + Gross)

\[ u(M) = \sqrt{0.0127^2 + 0.0127^2} = 0.01796 \]

Standard uncertainty of \( M \) std., \( u_M = \pm 0.01796 \) mg

Standard uncertainty of concentration of standard solution

\[ C = \frac{M}{V} \]

\[ u(c_{std}) = c_{std} \sqrt{\left(\frac{u_v}{V}\right)^2 + \left(\frac{u_M}{M}\right)^2} \]

\[ = 2.4921 \sqrt{\left(\frac{0.0234}{10.0}\right)^2 + \left(\frac{0.01796}{24.921}\right)^2} \]

\[ = 0.00610 \]

Standard uncertainty of \( C \) std., \( u(c_{std}) = \pm 0.00610 \) mg/ml

6. Repeatability

Assay: 4 replications, mean = 1488.95 unit/mg, SD = 6.37, RSD = 0.00428

Standard Uncertainty = standard deviation of mean = SD/\( \sqrt{n} \)

\[ = \pm 0.00428/\sqrt{4} = \pm 0.00214 \) ml

Standard uncertainty due to Repeatability, \( u_{(rep)} = \pm 0.00214 \) unit/mg
4. Combined Standard Uncertainty

<table>
<thead>
<tr>
<th>Source</th>
<th>value</th>
<th>Unit</th>
<th>Std. Uncert.</th>
<th>Rel. Std. Uncert.</th>
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<td>1 A std</td>
<td>1955917.3</td>
<td>-</td>
<td>15529.519</td>
<td>0.00079</td>
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<tr>
<td>2 A sam</td>
<td>1950585.0</td>
<td>-</td>
<td>5017.543</td>
<td>0.00257</td>
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<tr>
<td>3 M sam</td>
<td>126.9</td>
<td>mg</td>
<td>0.24</td>
<td>0.00189</td>
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<td>4 V sam</td>
<td>50.0</td>
<td>ml</td>
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<td>5 C std</td>
<td>2.4921</td>
<td>mg/ml</td>
<td>0.00610</td>
<td>0.00245</td>
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<tr>
<td>6 Repeatability</td>
<td>1.0</td>
<td>unit/mg</td>
<td>0.00214</td>
<td>0.00214</td>
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</table>

\[ Potency(\text{unit/mg}) = \frac{A_{\text{sam}} \times V_{\text{sam}} \times C_{\text{std}} \times P_{\text{std}}}{A_{\text{std}} \times M_{\text{sam}}} \]
\[ = \frac{195058507 \times 50.0 \times 2.4921 \times 1520}{1955197.3 \times 126.9} \]
\[ = 1488.44 \text{ unit/mg} \]

\[ u_c(\text{Potency}) = \sqrt{\left(u(A_{\text{sam}})^2 + \left(\frac{u(A_{\text{std}})}{A_{\text{std}}}\right)^2 + \left(\frac{u(M_{\text{sam}})}{M_{\text{sam}}}\right)^2 + \left(\frac{u(V_{\text{sam}})}{V_{\text{sam}}}\right)^2 + \left(\frac{u(C_{\text{std}})}{C_{\text{std}}}\right)^2 + u(\text{prec.})^2} \]
\[ = \sqrt{0.00257^2 + 0.00079^2 + 0.00189^2 + 0.00069^2 + 0.00245^2 + 0.00214^2} \]
\[ = 0.004677 \]

Combined standard uncertainty = ± 6.48 unit/mg

\[ u_c(\text{Potency}) = Potency \times 0.004677 \]
\[ = 1488.44 \times 0.004677 \]
\[ = 6.96 \]

Expanded Uncertainty

The expanded uncertainty, U(Potency_{\text{Pen V}}), is calculated by multiplying the combined standard uncertainty by a coverage factor of 2:

\[ U(\text{Potency}_{\text{Pen V}}) = 6.96 \times 2 = \pm 13.92 \text{ unit/mg} \]

The potency of the Pen. V is 1488.95 ±13.92 unit/mg
Spreadsheet calculation of uncertainty

<table>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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<td>PA sam</td>
<td>M sam</td>
<td>V sam</td>
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<td>Precision</td>
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**Diagram: u(y, x) unit/mg**

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