Contents

A. INTRODUCTION ..................................................................................... 2

B. EQUIPMENT ........................................................................................... 2

C. REAGENTS AND SOLUTIONS ............................................................... 3

D. STANDARD(S) ........................................................................................ 4

E. SAMPLE PREPARATION ........................................................................ 7

F. ANALYTICAL PROCEDURE .................................................................. 7

G. CALCULATIONS / IDENTIFICATION .................................................... 12

H. SAFETY INFORMATION AND PRECAUTIONS ................................. 13

I. QUALITY ASSURANCE PLAN .............................................................. 14

J. APPENDIX ............................................................................................. 14

K. APPROVALS AND AUTHORITIES ...................................................... 15
A. INTRODUCTION

1. Summary of Procedure

   Tissue samples are homogenized in 1% phosphate buffer, vortexed with acetonitrile, and centrifuged. After solvent evaporation, SPE cleanup (Strata X, 60 mg, 3 ml columns), and sample reconstitution, the penicillin in the samples is quantitated and confirmed by LC-MS/MS.

2. Applicability

   This method is suitable for the determination and confirmation of penicillin G in bovine kidney, liver and muscle at levels ≥ 25 ppb.

   Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

B. EQUIPMENT

   Note: Equivalent equipment may be substituted.

1. Apparatus
   a. Adjustable pipettors: Eppendorf 100 and 1000 µL (Brinkman Instruments Inc.)
   b. Class A volumetric pipettes: 3 and 4 mL (or 5 mL adjustable Eppendorf pipette)
   c. Spatulas: stainless steel or disposable.
   d. Analytical balance: 0.01 mg sensitivity (Mettler Toledo).
   e. Top loading balance(s): 0.01g and 0.001g sensitivities (Mettler Toledo)
   f. Class A volumetric flasks: amber glass, 10 and 100 mL.
   g. Centrifuge: equipped with a rotor with 15 mL centrifuge tube holders and capable of attaining 3000 g (~4,100 rpm).
   h. High-speed microcentrifuge: equipped with a fixed-angle rotor with 2 mL microcentrifuge tube holders and capable of attaining 16,000 g (~13,200 rpm).
   i. Disposable polypropylene conical centrifuge tubes: 15 mL (Corning brand obtained through Fisher Scientific Co), or equivalent tubes with leak-proof caps and graduations.
   j. Falcon round-bottom polypropylene 14-mL centrifuge tubes
   k. High-speed microcentrifuge filter tubes: modified nylon 0.2 µm, 500 µL (VWR).
   l. Solid phase extraction columns: Strata X, 33µm, 60 mg, 3 mL (Phenomenex).
m. Solvent evaporator: TurboVap LV with a 15 mL test tube rack (Zymark).

n. Solid phase extraction vacuum manifolds: 12 and 24-port (Supelco/Sigma-Aldrich).

o. Ultrasonic cleaner: Model 175D (Crest Ultrasonics Corp)

p. Vortex mixer: Vortex-Genie 2 mixer (Scientific Industries, Inc.).

q. Multi-tube vortexer: VX-2500 analog vortexer (VWR).

r. Reciprocating Shaker: Model 6010 or better, Eberbach Corporation.

s. Repipet dispensers: capable of dispensing variable volumes between 1 to 20 mL.

t. Graduated cylinder: 500 mL.

u. Autosampler vials: 2 mL, amber.

v. Freezer: capable of maintaining temperatures below -80°C for standards and extracts.

w. Beakers: glass, various sizes.

2. Instrumentation

a. LC system: ACQUITY UPLC Liquid Chromatograph (Waters) or Agilent 1100 (Agilent Technologies, Inc.) Model G1312A binary pumps and Model G1329A autosampler.


c. Liquid chromatography column: Polar-RP Synergi 2.5 µm, 20 x 2.0 mm (Cat. No.00M-4371-BO) with a guard column of the same packing 4 x 2.0 mm (Cat. No. AJ0-6075 and holder Cat. No. KJ0-4282) (Phenomenex). Do not use LC column (Cat. No. 00M-4371-BO-CE) because their casing is too thick and will not fit in many LC column compartments.

d. Pre-column filter: Upchurch filter assembly A-318 with fritz A-102x (Cole Palmer) is installed between the autosampler and the guard column.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependent on the expiration dates of the components used. The maximum length of time that a working reagent shall be used is 1 year unless the laboratory has produced extension data.

1. Reagents

a. Methanol (MeOH) – HPLC grade Burdick & Jackson.
2. Solutions
   a. 1% Phosphate buffer (pH~6.2)
      Dissolve 8.0 g of KH$_2$PO$_4$ and 2.0 g of K$_2$HPO$_4$ in a 1 L volumetric flask using Milli-Q-water as solvent. This solution may be stored for 2 months in a refrigerator. Bring to room temperature before using or store at room temperature for no longer than 1 week.

   b. Mobile Phase:
      20% ACN / 80% water solution that is also 0.1% formic acid
         Premixed:
         Mix 200 mL of ACN, 800 mL of Milli-Q-water and 1.0 mL of formic acid. This solution may be used for a maximum of two days at HPLC conditions (i.e. room temperature and open to atmosphere).
         Mixed by HPLC:
         0.1% aqueous formic acid:
         Add 0.5mL of formic acid to 500 mL of Milli-Q-water and mix. This solution may be used for one month at HPLC conditions.
         0.1% formic acid in acetonitrile:
         Add 0.5mL of formic acid to 500 mL of ACN and mix. This solution may be used for one month at HPLC conditions.

      Note: Only Premixed or mixed by HPLC is needed not both.

D. STANDARD(S)

   Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution is dependant on the expiration date of the components used. In-house prepared standards shall be assigned an expiration date that is no later than the expiration date of the earliest expiring component or no later than the stability stated in the method, whichever ends soonest. The maximum length of time that an in-house prepared standard shall be used is 1 year unless the laboratory has produced extension...
1. Standard Information
   b. Penicillin G-d7 potassium salt – (Toronto Research Chemicals).

2. Preparation of Standard Solution(s)
   a. Internal Standard Stock Solution
      Weigh ~ 2 mg of penicillin G-d7 potassium in a weighing boat and record the mass to the nearest 0.01 mg. Transfer to a 10-mL volumetric flask using Milli-Q water and mix well. Correct for the purity and salt form.

      Concentration of PenG-d7 in mg/mL = \(\frac{\text{mass in mg} \times \text{purity factor} \times 341.4}{10 \times 379.5}\)

      Subdivide in aliquots of ~ 0.5 mL using amber vials and store in a -80°C freezer. The stability of this solution is two days when stored in a refrigerator, two weeks in a -20°C freezer, and one year in a -80°C freezer.

   b. Preparation of Internal Standard Working Solution (0.20 ng/µL Penicillin G-d7):
      Calculate the volume of the penicillin G-d7 stock solution (D.2.a) that is required to prepare 100 mL of a solution at concentration 0.20 ng/µL. Accurately measure and transfer the volume of the concentrated stock solution into a 100 mL volumetric flask using an adjustable micropipette. Bring to volume with Milli-Q water and mix. Subdivide in aliquots of ~ 5 mL and store in a -80°C freezer. The stability of this solution is 6 months when stored at -80°C. Thaw one vial of this internal standard working solution on each day of analysis and discard any remaining solution at the end of the workday.

   c. Penicillin G Stock Solution ~ 1 mg/mL:
      Weigh ~ 11.1 mg of penicillin G potassium in a weighing boat and record the mass to the nearest 0.01 mg. Transfer to a 10 mL volumetric flask using Milli-Q water and mix well. Correct the concentration for purity and acid form.

      Concentration of Penicillin in mg/mL = \(\frac{\text{mass in mg} \times \text{purity factor} \times 334.4}{10 \times 372.5}\)

      Subdivide in aliquots of ~ 0.5 mL using amber vials and store in a -80°C freezer. The stability of this solution is two days in a refrigerator, two weeks in a -20°C freezer, and 6 months in a -80°C freezer.
d. Preparation of Penicillin G Working Solutions for Assaying Kidney or Liver Samples:

The working solutions are prepared each day from a freshly thawed vial of penicillin stock solution (1 mg/mL). Calculate the volume of the stock solution that is required to prepare 100 mL at a concentration of 1.00 ng/µL. Accurately measure and transfer the volume (~100 µL) of the concentrated stock solution into a 100 mL amber volumetric flask using an adjustable micropipette. Bring to volume with Milli-Q water, mix, and label it Stock A. Prepare aqueous working penicillin G solutions at Levels 1 – 6 as described in Table 1. Use 10-mL amber volumetric flasks, 100 and 1000 µL adjustable micropipettes, and class A volumetric pipettes (or a 5 mL adjustable pipette). For example, to prepare a working standard at Level 3 (0.10 ng/µL), transfer 1.00 mL of Stock A to a 10 mL volumetric flask and dilute to the mark with Milli-Q water. All working solutions are discarded at the end of the day.

Table 1 Preparation of working solutions for kidney and liver assay

<table>
<thead>
<tr>
<th>Solution Level</th>
<th>Volume of Flask (mL)</th>
<th>Stock A (mL to use)</th>
<th>Conc. (ng/µL)</th>
<th>Fort. Equiv. Conc. (ng/g in kidney or liver sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>10</td>
<td>0.300</td>
<td>0.030</td>
<td>15</td>
</tr>
<tr>
<td>L2</td>
<td>10</td>
<td>0.500</td>
<td>0.050</td>
<td>25</td>
</tr>
<tr>
<td>L3</td>
<td>10</td>
<td>1.00</td>
<td>0.10</td>
<td>50</td>
</tr>
<tr>
<td>L4</td>
<td>10</td>
<td>2.00</td>
<td>0.20</td>
<td>100</td>
</tr>
<tr>
<td>L5</td>
<td>10</td>
<td>5.00</td>
<td>0.50</td>
<td>250</td>
</tr>
<tr>
<td>L6</td>
<td>As is</td>
<td></td>
<td>1.0</td>
<td>500</td>
</tr>
</tbody>
</table>

e. Preparation of Penicillin G Working Solutions for Assaying Muscle Samples:

The working solutions are prepared each day from a freshly thawed vial of stock solution (1 mg/mL). Calculate the volume of the stock solution that is required to prepare 100 mL of penicillin G solution at a concentration of 2.50 ng/µL. Accurately measure and transfer the volume of the concentrated stock solution (~250 µL) into a 100 mL amber volumetric flask using an adjustable micropipette. Bring to volume with Milli-Q water, mix, and label it Stock B. Prepare aqueous working penicillin G solutions at Levels 1 – 6 as described in Table 2.
Table 2: Working Solutions for Muscle assay

<table>
<thead>
<tr>
<th>Solution Level</th>
<th>Volume of Flask (mL)</th>
<th>Stock B (mL to use)</th>
<th>Conc. (ng/µL)</th>
<th>Fort. Equiv. Conc. (penicillin ng/g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>10</td>
<td>0.300</td>
<td>0.075</td>
<td>15</td>
</tr>
<tr>
<td>L2</td>
<td>10</td>
<td>0.500</td>
<td>0.125</td>
<td>25</td>
</tr>
<tr>
<td>L3</td>
<td>10</td>
<td>1.00</td>
<td>0.25</td>
<td>50</td>
</tr>
<tr>
<td>L4</td>
<td>10</td>
<td>2.00</td>
<td>0.50</td>
<td>100</td>
</tr>
<tr>
<td>L5</td>
<td>10</td>
<td>5.00</td>
<td>1.25</td>
<td>250</td>
</tr>
<tr>
<td>L6</td>
<td>As is</td>
<td>2.5</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

f. Preparation of External Calibration curve

To prepare 1 mL of the individual calibration standards, pipette 50µL of the penicillin-d7 internal standard working solution (0.20 ng/µL) into six amber autosampler vials. Add 50µL of one of the penicillin working standards (L1 to L6) to the autosampler vials followed by 900 µL of Milli-Q water and vortex- mix. In the extraction procedure, the samples are fortified with twice the amount of the penicillin and internal standards (100 µL/each) but the final extracts are diluted to 2 mL. This approach facilitates the preparation of the calibration standards and injects in the mass spectrometer the same amounts of IS and analyte for both samples and standards.

E. SAMPLE PREPARATION

Control and tissue samples are previously homogenized with a blender and stored in a -10°C freezer.

Note: One can strive for temperatures < -10°C since penicillin in tissue is unstable even at -30°C.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

a. For kidney and liver weigh 0.200 ± 0.010 g known blank tissue (recovery, blank, and check sample, if needed) and samples while still partially frozen into polypropylene centrifuge tubes (Penicillin in tissue undergoes fast degradation at room temperature).
b. For muscle weigh 0.500 ± 0.010 g known blank tissue (recovery, blank, and check sample, if needed) and samples while still partially frozen into polypropylene centrifuge tubes (Penicillin in tissue undergoes fast degradation at room temperature).

Note: Must use appropriate tissue controls with each sample batch.

c. Fortification:
   i. For kidney or liver positive control fortify with 100 µL of L3 working standard (Table 1) and check sample, if needed.
   ii. For muscle positive control fortify with 100 µL of L3 working standard (Table 2) and check sample, if needed.

Note: Fortify samples while still partially frozen and proceed immediately to the vortex step. The internal standard corrects for the degradation of penicillin in the samples only after the samples are vortexed and shaken. Once the extraction procedure is started it must be completed in a single day.

d. Add 100 µL of the 0.20 ng/µL penicillin G-d7 working IS to all tubes.

2. Extraction Procedure
   a. Add 2.0 mL of 1% phosphate buffer to all tubes (samples and controls).
   b. Bring the total volume of all tubes to the 5 mL mark with ACN.
   c. Shake forcefully for 10 min using a reciprocal shaker.
   d. Centrifuge 10 min at 3000 g (~4100 rpm). Pour the supernatants into 15 mL graduated polypropylene centrifuge tubes.

Note: Begin evaporation.

e. Add 2 mL phosphate buffer and 3 mL of ACN to the tissue pellet. Break the pellet and shake forcefully for 5 min using a reciprocal shaker.
   f. Centrifuge as described in step d and combine with the first supernatants.
   g. Evaporate down to ~ 3.5 mL using a nitrogen stream in a 40 ± 2°C water bath.

Note: Penicillin G decomposes at high temperatures and not evaporating all the ACN reduces the adsorption of penicillin to the SPE sorbent. Evaporating the ACN at a higher temperature or incomplete evaporation of the ACN will both cause low recoveries.
   h. Add water up to the 5-mL mark and vortex 1 minute.
   i. Centrifuge extract for 10 min. at room temperature and 3000 g (~4100 rpm).
   j. Condition Strata X, 60 mg, 3 mL SPE cartridges with 2.0 mL MeOH, followed by 3.0 mL phosphate buffer.
k. Load the extracts onto the SPE cartridges, ~0.5 mL/min. Do not apply vacuum.

l. Rinse the walls of the sample tubes with 2.0 mL of phosphate buffer. Tilt and rotate the tube while adding the buffer with a dropper to avoid dispersing the residue. Load the rinse onto the SPE cartridges and discard the sample tubes.

m. Wash SPE column with 2 mL more of phosphate buffer. After the column wash, apply vacuum to dry the SPE columns.

n. Elute analytes from the SPE columns with 3 mL of 100% ACN into clean 15 mL polypropylene centrifuge tubes. Apply vacuum to elute the last drops of ACN from SPE columns.

o. Add 2 mL of water and evaporate the ACN down to just below 2.0 mL using the Zymark evaporator. (Adding water prevents analyte adsorption to the tube walls).

p. Bring the volume in all tubes to the 2.00 mL mark with Milli-Q water and vortex 1 min.

q. Pour ~ 500 µL of the samples into high-speed 500 µL centrifuge filter tubes.

r. Centrifuge in a microcentrifuge for 5 min at ~6,150 g (~5,000 rpm).

s. Transfer the supernatants into amber glass autosampler vials using a dropper. Note: To avoid cross contamination, do not pour samples from the microcentrifuge tubes into autosampler vials. Residues of analyte in the microcentrifuge tube holders adhere to the lip of the microcentrifuge tubes and are transferred to the autosampler vials when samples are poured instead of pipetted out.

t. Inject 10 µL into the LC-MS/MS. Extracts may be stored until analysis at <-10°C for one week, at -80°C for 2 months, and no more than 12 hours in a refrigerator.

3. Instrumental Settings

   Note: The instrument parameters may be optimized to ensure system suitability.

   a. UHPLC Conditions

      | Column         | Phenomenex Polar-RP Synergi – 2.5 µm, 20 x 2.0 mm |
      | Guard Column   | Phenomenex Polar-RP Synergi – 4 µm, 4 x 2.0 mm   |
      | Temperature    | 30°C                                                 |
      | Autosampler temperature | 10°C                                              |
      | Injection volume (µL) | 10                                                  |
      | Run time (min)  | 6                                                   |
      | Flow (mL/min)   | 0.300                                               |
      | Mobile phase    | 20% ACN/80% water solution that is also 0.1% formic acid |
b. Quattro Micro Parameters

Tune the mass spectrometer using ~ 20 µg/mL solutions of the individual standards infused into the mobile phase at a flow of 5µL/min. The precursor ions used for collision-induced dissociation (CID) and the selected reaction monitored transitions (SRM) for the LC-MS/MS analysis listed in Table 4.

Table 4 Analyte dependent parameters

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Precursor Ion (Da)</th>
<th>Product Ion (Da)</th>
<th>Cone (V)</th>
<th>Collision Energy (eV)</th>
<th>Dwell Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>335.10</td>
<td>160.10</td>
<td>17</td>
<td>11</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>176.15</td>
<td>17</td>
<td>12</td>
<td>0.300</td>
</tr>
<tr>
<td>Penicillin G-d7</td>
<td>342.20</td>
<td>160.20</td>
<td>16</td>
<td>11</td>
<td>0.200</td>
</tr>
</tbody>
</table>

Quantitate using the signal area from the precursor ion fragmenting to the most abundant product ion (m/z 176) using multiple reaction monitoring.

Tune Parameters

- RF Lens (V): Positive
- Capillary (kV): 1.72
- Cone (V): Table 4
- Extractor (V): 2.00
- RF Lens (V): 0.1
- Source Temperature (°C): 125
- Desolation Temperature (°C): 375
- Cone Gas Flow (L/Hr): 10
- Desolation Gas Flow (L/Hr): 400

Analyzer Parameters

- Resolution: 13.0
- HM 1 Resolution: 13.0
- Ion Energy 1: 2.3
- Entrance: -5
Collision Energy Table 4
Exit 1 1
LM 2 Resolution 13.0
HM 2 Resolution 13.0
Ion Energy 2 2.3
Multiplier (V) 650
Gas Cell Pirani (mbar) \(3.0 \times 10^3\)
Inter Channel Delay (s) 0.020
Inter Scan Delay (s) 0.100

c. Instrument Process
   i. Equilibrate the collision gas and the electronics of the mass spectrometer for an hour.
   ii. Equilibrate the LC column with the mobile phase for at least one hour prior to injecting samples.
   iii. Inject one blank and Level 1 standard. Use the first injection of standard Level 1 to test the instrument response but don’t use it for quantitation or confirmation purposes.

d. Example Injection sequence
   Inject Level-1 to Level-6 calibration standards, a solvent blank, the negative control, the fortified, incurred or unknown samples, a solvent blank, and a repeat of the calibration standards.

e. Example instrument cleanup
   Remove the formic acid and other retained materials from the column and the sample loop after the batch as follows. Make five 10-µL injections of water using a mobile phase of 50% ACN/50% Milli-Q water at a flow of 0.300 mL/min and a run time of 6 minutes.

4. Sample Set
   a. Negative Control
   b. Positive Controls/ Recoveries
   c. Check sample if necessary
   d. Up to 22 Samples
G. CALCULATIONS / IDENTIFICATION

1. Calculations/ Criteria for determinative analysis in Bovine Tissue
   a. Use penicillin G-d7 as internal standard to prepare a linear calibration curve that includes the two bracketing series of standards. Use 1/x weighting and plot the response of the analyte in each standard versus the nominal concentration.

   \[
   \text{Response} = \frac{\text{Analyte peak area} \times \text{I.S. Concentration}}{\text{I.S. peak area}}
   \]

   b. Calculate the linear regression parameters for the calibration curve and interpolate the concentrations of the samples from the regression parameters. Do not use zero as a regression data point.

   c. Export the processed data into Microsoft Excel or equivalent spreadsheet program for further calculations. The experimental analyte concentrations (except fortified samples) are corrected for the mass difference of the individual samples to the nominal mass of sample used to calculate the concentration of the analytes in the calibration standards. Use the equation:

   \[
   \text{Mass corrected conc (ng/g)} = \frac{\text{experimental conc.} \times \text{nominal mass}}{\text{Mass of sample in grams}}
   \]

   The accuracy of fortified samples is calculated using the equation:

   \[
   \text{Accuracy (\%)} = \frac{\text{experimental concentration} \times 100}{\text{Nominal concentration}}
   \]

   d. The negative control must be < 5% of the recovery standard.

   e. The deviation of at least five of the six standards in each bracketing calibration curve is within 10% of its nominal value. No one standard can have an average deviation larger than 15% of its nominal value.

   f. The coefficient of determination \((r^2)\) for the calibration curve is > 0.99.

2. Calculations / Criteria for Confirmation Analysis in Bovine Tissue
   a. Integrate the two product-ion chromatograms for each analyte. Inspect the automatic peak integrations and manually correct if base-to-base integration was not achieved.
b. Calculate the ion-abundance ratio from the two monitored ions using the most intense ion as the denominator. The ion-abundance ratio is always less than 1.

\[
\text{Ion abundance Ratio} = \frac{\text{Area of less intense product ion}}{\text{Area of most intense product ion}}
\]

The ion-abundance ratio of the analyte in the samples is arithmetically within 10% of the average abundance ratio of the standards. For example, if the average ion-abundance ratio of the standards is 0.77, the acceptance range for the samples is 0.67 – 0.87.

c. Compare the retention time of each individual product ion in the unknowns and fortified samples to the corresponding average retention times in the standards. The retention time of each of the two product ions of the analyte in the samples must agree within ± 3% (relative) its mean retention time in the standards.

d. The two product ions associated with analyte are present and have a signal to noise ratio > 10:1. This may be verified by visual inspection.

e. The negative control must be < 5% of the recovery standard.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Protective clothing, eyewear, and gloves, where applicable.

2. Hazards

<table>
<thead>
<tr>
<th>Procedure Step</th>
<th>Hazard</th>
<th>Recommended Safe Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>Flammable, toxic, may be fatal if inhaled or absorbed.</td>
<td>Use only in a fume hood. Keep away from flame or heat.</td>
</tr>
<tr>
<td>Methanol</td>
<td>Flammable, harmful if swallowed.</td>
<td>Use only in a fume hood. Keep away from flame or heat.</td>
</tr>
<tr>
<td>Potassium Phosphate</td>
<td>May cause eye, skin, and respiratory tract irritation</td>
<td>Use only in a fume hood. Wear suitable protective clothing, gloves and safety glasses.</td>
</tr>
<tr>
<td>Formic acid</td>
<td>Corrosive, Caustic</td>
<td>Use only in a fume hood. Wear suitable protective clothing, gloves and safety glasses.</td>
</tr>
<tr>
<td>Penicillin Std</td>
<td>Some individuals may have allergic reactions.</td>
<td>Use only in a fume hood. Wear protective clothing, gloves and safety glasses.</td>
</tr>
</tbody>
</table>
3. Disposal Procedures
Follow local, state and federal guidelines for disposal.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Tissue</th>
<th>Analytical Range</th>
<th>Acceptable Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>Kidney</td>
<td>15 – 400 ng/g (ppb)</td>
<td>80 - 110</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Liver</td>
<td>30 – 1200 ng/g (ppb)</td>
<td>80 -110</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Muscle</td>
<td>5 – 200 ng/g (ppb)</td>
<td>80 -110</td>
</tr>
</tbody>
</table>

2. Critical Control Points and Specifications
None

3. Intralaboratory Check Samples
a. System, minimum contents.
   i. Frequency: One per week per analyst when samples analyzed.
   ii. Records are to be maintained.

b. Acceptability criteria.
   Refer to I. 1.
   If unacceptable values are obtained, then:
   i. Investigate following established procedures.
   ii. Take corrective action as warranted.

4. Sample Condition upon Receipt – Frozen or cool.

J. APPENDIX

1. References
   Lopez, Mayda, and Smith, Shani , LC-MS/MS Determination and Confirmation of Penicillin G in Bovine Tissue and Plasma, CVM Office of Research, Division of Residue Chemistry 419.04, version 1.0, pp 1-26 (2010).
K. APPROVALS AND AUTHORITIES


2. Issuing Authority: Director, Laboratory Quality Assurance Division.