Vitamin A - Test Portion Comparison

Michele Swarbrick
Recap: FSE Relation to Sample Mass

Calculations based on liberated vitamin beadlets based on theory of sampling equations found in GOODTestPortions

Example

<table>
<thead>
<tr>
<th>Premix A, 1037000 IU/g, 31.11 % of Vit A in product, particle size 405um</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conc. of Vit A in feed</strong></td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>10000 IU/lb (0.000661%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>100000 IU/lb (0.006614%)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Recap: Samples Selected for Experiment

Three commercial feed samples with vitamin A levels of 10,000 IU/lb, 12,500 IU/lb and 100,000 IU/lb were purchased by Nancy Thiex
Recap: Samples Selected for Experiment

- **Sample 1:** Poultry Conditioner with Vitamin A claim of 10,000 IU/lb
  
  This consists of small pellets that are composed of corn, soybean meal, wheat middlings, DDG, fishmeal, flaxseed meal, added lysine and methionine and minerals.

- **Sample 2:** Texturized Medicated Feed with Vitamin A claim of 12,500 IU/lb
  
  This is a mix of pellets (which contain the vitamin A) and grains which are molasses coated. Pelleted portion = 49.4%.

- **Sample 3:** Mineral with Vitamin A claim of 100,000 IU/lb
Sample Comminution and Mass Reduction

• Prepared by Lawrence Novotny

- Good Test Potions principles were used throughout all steps.
- From each bag, 1800 grams was removed for the lab sample.
- Entire lab sample was comminuted using a centrifugal mill through a 1 mm screen.
- Comminuted material was mass reduced using a 8 port rotary splitter.
  - Seven bottles of the 1st mass reduction were combined and further reduced until 100 g test portions obtained.
  - Remaining bottle from 1st mass reduction further reduced until 10 g test portions obtained.
- Test portions transferred to zip-lock plastic bag and the portion weight was recorded.
- Test portions shipped frozen to MN Dept of Ag and stored at -30C until analysis.
Densities of Sample Materials

Determined by Lawrence Novotny

- Additional portion of each sample comminuted.
- Aliquot of the ground test material was transferred to a dry tared 100 mL graduated cylinder. The volume and mass of the material was recorded. Repeated 2 more times using a different aliquot of the test material.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Avg Density (g/ml):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1: Poultry Conditioner</td>
<td>0.4599</td>
</tr>
<tr>
<td>Sample 2: Texturized Medicated Feed</td>
<td>0.4927</td>
</tr>
<tr>
<td>Sample 3: Mineral</td>
<td>0.9506</td>
</tr>
</tbody>
</table>
Analysis Experiment Design

For each sample

- 16 test portions at 10 g (times 3 samples for total of 48 test portions)
- 16 test portions at 100 g (times 3 samples for total of 48 test portions)

Due to limitations of equipment and time, it was decided to analyze, eight portions of 10 g and eight portion of 100 g (10 g x 8 and 100 g x 8) of one product on day one. The second eight portions within a few days, for a total of six analytical runs.
## Saponification and Neutralization

<table>
<thead>
<tr>
<th></th>
<th>10 g Test Portions</th>
<th>100 g Test Portions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extraction Container</strong></td>
<td>250 ml Amber HDPE</td>
<td>1000 ml Amber HDPE</td>
</tr>
<tr>
<td><strong>Ethanol/Pyrogallol</strong></td>
<td>80 mL</td>
<td>320 mL</td>
</tr>
<tr>
<td><strong>KOH</strong></td>
<td>20 mL</td>
<td>80 mL</td>
</tr>
</tbody>
</table>
| **Pyrene ISTD**         | • 0.5 ml for Standards, QC, Samples 1 & 2  
                          • 2 ml for Sample 3 | • 4 ml for Standards, QC, Samples 1 & 2  
                          • 20 ml for Sample 3 |
| **Acetonitrile / Acetic Acid** | 50 mL | 200 mL |
| **Total Volume**        | ~ 150 mL Solvent   | ~ 600 mL Solvent    |
Analysis

- Samples saponified overnight on reciprocating shaker.
- Saponified samples neutralized next morning.
- Aliquot of neutralized sample centrifuged and diluted as needed with acetonitrile and analyzed on HPLC-DAD.
- Pyrene ISTD used to account for variations in adding solvents from saponification, neutralization, and dilution steps.
- Analysis of all 96 test portions completed within 2 week timeframe.
## Results

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Poultry Conditioner</th>
<th>Texturized Medicated Feed</th>
<th>Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 g portions</td>
<td>100 g portions</td>
<td>10 g portions</td>
</tr>
<tr>
<td>1</td>
<td>2772</td>
<td>2675</td>
<td>9467</td>
</tr>
<tr>
<td>2</td>
<td>2372</td>
<td>2607</td>
<td>8549</td>
</tr>
<tr>
<td>3</td>
<td>2427</td>
<td>2564</td>
<td>11145</td>
</tr>
<tr>
<td>4</td>
<td>2211</td>
<td>2621</td>
<td>6866</td>
</tr>
<tr>
<td>5</td>
<td>3055</td>
<td>2822</td>
<td>10798</td>
</tr>
<tr>
<td>6</td>
<td>3538</td>
<td>2878</td>
<td>10288</td>
</tr>
<tr>
<td>7</td>
<td>2982</td>
<td>2916</td>
<td>15493</td>
</tr>
<tr>
<td>8</td>
<td>3308</td>
<td>2686</td>
<td>12041</td>
</tr>
<tr>
<td>9</td>
<td>3092</td>
<td>2234</td>
<td>10289</td>
</tr>
<tr>
<td>10</td>
<td>2616</td>
<td>2490</td>
<td>8214</td>
</tr>
<tr>
<td>11</td>
<td>2577</td>
<td>2337</td>
<td>11753</td>
</tr>
<tr>
<td>12</td>
<td>2107</td>
<td>2396</td>
<td>14212</td>
</tr>
<tr>
<td>13</td>
<td>3131</td>
<td>2803</td>
<td>10397</td>
</tr>
<tr>
<td>14</td>
<td>2679</td>
<td>2371</td>
<td>13068</td>
</tr>
<tr>
<td>15</td>
<td>2606</td>
<td>2662</td>
<td>7410</td>
</tr>
<tr>
<td>16</td>
<td>2444</td>
<td>2449</td>
<td>6407</td>
</tr>
<tr>
<td>Average</td>
<td>2745</td>
<td>2594</td>
<td>10400</td>
</tr>
<tr>
<td>Std Dev</td>
<td>392</td>
<td>197</td>
<td>2491</td>
</tr>
<tr>
<td>%RSD</td>
<td>14%</td>
<td>8%</td>
<td>24%</td>
</tr>
</tbody>
</table>
## Comparison of Actual to Theoretical Results

<table>
<thead>
<tr>
<th></th>
<th>Poultry Conditioner</th>
<th>Texturized Medicated Feed</th>
<th>Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10,000 IU/lb</td>
<td>12,500 IU/lb</td>
<td>100,000 IU/lb</td>
</tr>
<tr>
<td>%RSD:</td>
<td>14.3%</td>
<td>24.0%</td>
<td>24.0%</td>
</tr>
<tr>
<td>%FSE:</td>
<td>24.8%</td>
<td>22.2%</td>
<td>7.0%</td>
</tr>
</tbody>
</table>

**Theoretical based on equations in GOOD Test Portions**

<table>
<thead>
<tr>
<th></th>
<th>10 g portions</th>
<th>100 g portions</th>
<th>10 g portions</th>
<th>100 g portions</th>
<th>10 g portions</th>
<th>100 g portions</th>
</tr>
</thead>
<tbody>
<tr>
<td>%RSD:</td>
<td>7.6%</td>
<td>10.3%</td>
<td>10.1%</td>
<td>2.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%FSE:</td>
<td>7.8%</td>
<td>7.0%</td>
<td>7.8%</td>
<td>2.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What’s Next?

- Data and theoretical calculations show a 100 g test portion is needed
- How to Deal with 100 gram Test Portion?
  - Saponify 100g and take portion of saponified sample for analysis
  - Dissolve encapsulation with enzyme(s), then homogenize and remove portion for saponification
  - CryoMill 100 g, then remove a small portion for analysis
  - Supercritical Fluid Extractor for 100 g
  - Analyze 10 x 10 g portions and average for single result
  - Other Ideas??
thank you!

- Nancy Thiex
- Lawrence Novotny
- Dorota Inerowicz
- Ken Riter