

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

Premix A, 1037000 IU/g, 31.11 % of Vit A in product, particle size 405um			
Conc. of Vit A in feed	Sample portion m_s (g)	S^2_{FSE}	FSE (%)
10000 IU/lb (0.000661%)	100	0.0061518	7.8
	10	0.0615183	24.8
100000 IU/lb (0.006614%)	100	0.0006151	2.5
	10	0.0061517	7.8

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 Portions 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.

Sample Type	Avg Density (g/ml):
Sample 1: Poultry Conditioner	0.4599
Sample 2: Texturized Medicated Feed	0.4927
Sample 3: Mineral	0.9506

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

	10 g Test Portions	100 g Test Portions
Extraction Container	250 ml Amber HDPE	1000 ml Amber HDPE
Ethanol/Pyrogallol	80 mL	320 mL
KOH	20 mL	80 mL
Pyrene ISTD	<ul style="list-style-type: none">• 0.5 ml for Standards, QC, Samples 1 & 2• 2 ml for Sample 3	<ul style="list-style-type: none">• 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

Replicate	Poultry Conditioner		Texturized Medicated Feed		Mineral	
	10 g portions	100 g portions	10 g portions	100 g portions	10 g portions	100 g portions
1	2772	2675	9467	11100	74722	80085
2	2372	2607	8549	8364	74249	77638
3	2427	2564	11145	9884	83887	78626
4	2211	2621	6866	10364	79930	80289
5	3055	2822	10798	8907	81818	76638
6	3538	2878	10288	8908	92170	81668
7	2982	2916	15493	7581	62938	78808
8	3308	2686	12041	8701	83617	81938
9	3092	2234	10289	10884	77302	77468
10	2616	2490	8214	8845	68506	77809
11	2577	2337	11753	9852	84238	81678
12	2107	2396	14212	8606	76527	82106
13	3131	2803	10397	8869	86567	77550
14	2679	2371	13068	8246	93336	80500
15	2606	2662	7410	9136	83175	80438
16	2444	2449	6407	10104	92568	79489
Average	2745	2594	10400	9272	80972	79546
Std Dev	392	197	2491	958	8197	1744
%RSD	14%	8%	24%	10%	10%	2%

Comparison of Actual to Theoretical Results

	Poultry Conditioner 10,000 IU/lb		Texturized Medicated Feed 12,500 IU/lb		Mineral 100,000 IU/lb	
	10 g portions	100 g portions	10 g portions	100 g portions	10 g portions	100 g portions
%RSD:	14.3%	7.6%	24.0%	10.3%	10.1%	2.2%

Theoretical based on equations in GOOD Test Portions

Calculated %FSE:	24.8%	7.8%	22.2%	7.0%	7.8%	2.5%
------------------	-------	------	-------	------	------	------

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