AOAC Agricultural Materials Task Force February 23, 2010
Sub-Committee on Feed Additives and Contaminants - Second Draft

Project: A method for the determination of total minerals in animal feed, feed ingredients, forage, grain and pet food.

Method Needs Statement and Validation Criteria

1. Method Needs Statement

Inorganic elements found in Earth's crust are often referred to as minerals. Some minerals are essential for health and productivity of animals and have well-defined nutritional and biochemical roles. Many other minerals naturally occur at trace levels in the foods and tissues of animals but are not typically suspected to play a useful nutritional purpose and are considered incidental contaminants. However, all minerals whether essential or nonessential, can adversely affect an animal when amounts in the diet and water become excessive, so the prevention of mineral toxicosis is a fundamental part of animal nutrition and management. The single Official AOAC multi-element method is AOAC Method 968.08, atomic absorption spectroscopy method for copper, iron, manganese, zinc and calcium. A need exists for a method that includes additional analytes and potentially additional technologies. A versatile method that would allow for optional technologies is desirable. The desired method should apply to feed and feed ingredients of animal and plant origin, excluding inorganic mineral mixes.

2. Performance Characteristics

The following performance characteristics must be demonstrated by the method.

2.1 Selectivity (Specificity)
The method should be capable of detecting as many of the high priority elements of concern as possible. Elements of low priority should be included if possible; however when a compromise in the multi-analyte method is necessary it should favour the high priority elements. The method must be capable of distinguishing these elements from each other as well as from other substances within grains, forages and feedstuffs. It must be demonstrated to be free of interference from the other analytes included in the method over the concentration ranges of the method.

2.2 Limit of Quantitation (LOQ) Levels:
The method should aim to quantify as many of the specified elements in feeds, forage, grain and other feed ingredients as possible at or below the LOQ levels indicated in Table 1. It is recognized that these LOQ values are to be used as *target quantitation levels* and may not be achievable for all elements.

The limits of quantitation are based on the lowest Maximum Tolerable Level (MTL) as published in the 2005 NRC Mineral Tolerances in Animal Health for various species. For optimal quantitation, each LOQ was designated as 1/10 of the lowest MTL. For example if the MTL is 100 mg/kg in feed, a target LOQ is 10 mg/kg. For essential elements, the target LOQ was equivalent to 1/10 the concentration that would be considered deficient in feed, if that level is lower than 1/10 the lowest MTL.

2.3 Operational range:
The method should be capable of detecting and quantifying as many of the specified elements as possible over the ranges indicated in Table 1. The upper range of the method was selected as twice the highest MTL.

2.4 Accuracy:
The method should demonstrate accuracy as specified in Table 1 and Figure 1. This accuracy requirement must be met by measuring naturally incurred or fortified elements in grains, forages and feeds, at the midpoint of the operational range as well as the LOQ.

AOAC's Single Lab Validation document recommends that accuracy be measured at "1x or 2x the expected concentration". For the elements of study, the concentration range may be great. Therefore, for the purposes of this document, the middle of the operational range and 2x the LOQ may be considered the "expected concentrations". Therefore, accuracy measurements should be made at both of these concentration levels (see Table 1).

2.5 Repeatability

The coefficient of variation will depend upon the target quantitation level. The repeatability coefficient of variation (CV_r) will vary depending on the concentration of the element in feed matrix. The method should demonstrate repeatability as specified in Table 1 and Figure 2.

Concentration	CV_r (HCV)	RSD = $C^{-0.15}$
10%	<3	1.5
5%	<3	
1 %	<4	2
5000 mg/kg	<5	
1000 mg/kg	<6	3
500 mg/kg	<7	
100 mg/kg	<8	4
50 mg/kg	<9	5
10 mg/kg	<12	6
5 mg/kg	<13	7
1 mg/kg	<16	8
0.5 mg/kg	<18	9
0.1 mg/kg	<22	11
0.05 mg/kg	<25	13
0.01 mg/kg	<32	16

Figure 2. Repeatability coefficients for specified concentrations

The repeatability shall be measured by multiple analyses of naturally incurred or added elements in grains, forages and feeds, at both the midrange and at 2x the LOQ.

2.6 Reproducibility

As with repeatability, the variation will depend upon the target quantitation level and the reproducibility coefficient of variation (CV_R) will vary depending upon the concentration of the element in the feed matrix. The reproducibility shall be measured by analysing several naturally incurred or added elements in grains, forages and feeds, at both the midrange and at 2x the LOQ. The reproducibility should be approximately twice the repeatability as specified in Figure 2 (CV_R = 2x HCV).

3. Special consideration criteria

In addition to LOQs below the lower limit of the operational range, as described in Table 1, candidate methods will also be evaluated against subjective criteria including method simplicity, method costs, use of commercially available consumables and common laboratory instrumentation, and existence of in-house, single-laboratory validation.

4. Method validation protocol

The method is to be rugged and robust and critical parameters are to be identified and controlled. The method performance criteria are to be defined. A familiarization plan is to be suggested which will demonstrate that the laboratory analyst can capably perform the method prior to analyzing samples. In addition, a quality control plan is to be suggested along with warning and out of control limits.

5. Prospective technologies

At this time, two technologies that may be able to address the method needs have been identified. They are 1) ICP-OES and 2) ICP-MS. Element specific technologies may also be required, for example, a mercury analyzer. Optimal digestion or solubilization techniques will vary by element. While most elements in most feed matrices can be accomplished with a single acid digestion, others elements will require a specific solubilization techniques (such as alkaline digestion). Some elements, such as the halides, mercury and selenium will not be amenable to multi-element methods.

Table 1. Recommended Method Performance Characteristics:

	Target Concentration, mg/kg		Accuracy, %		Repeatability, % (CV_r)		Reproducibility, % (CV_R)	
High Priority Elements	**LOQ**	**Operational Range**	**at 2x LOQ**	**at midrange**	**at 2x LOQ**	**at midrange**	**at 2x LOQ**	**at midrange**
Aluminum,	20	20 - 2000	80 – 115	90 – 108	< 9	< 6	< 18	< 12
Arsenic	0.2	0.2 - 60	70 – 125	80 – 115	< 18	< 10	< 36	< 20
Boron,	15	15 - 300	80 – 115	85 – 110	< 9	< 8	< 18	< 16
Cadmium	0.05	0.05 - 20	75 – 120	80 – 115	< 22	< 12	< 44	< 24
Calcium	50	50 - 30000	85 – 110	92 – 105	< 8	< 4	< 16	< 8
Chromium	0.02	0.02 - 60000	70 – 125	92 – 105	< 25	< 4	< 50	< 8
Cobalt	0.01	0.01 - 50	70 – 125	80 – 115	< 30	< 10	< 60	< 20
Copper	0.4	0.4 - 1000	75 – 120	90 – 108	< 16	< 7	< 32	< 14
Fluorine	0.03	0.03 - 300	70 – 125	85 – 110	< 25	< 8	< 50	< 16
Iodine	0.01	0.01 - 800	70 – 125	85 – 110	< 30	< 7	< 60	< 14
Iron	5	5 - 6000	80 – 115	90 – 108	< 12	< 6	< 24	< 12
Lead	0.5	0.5 - 200	75 – 120	85 – 110	< 16	< 8	< 32	< 16
Magnesium	60	60 - 16000	85 – 110	92 – 105	< 8	< 4	< 16	< 8
Manganese	1	1 - 4000	75 – 120	90 – 108	< 15	< 6	< 30	< 12
Mercury	0.01	0.01 – 4	70 – 125	75 – 120	< 30	< 15	< 60	< 30
Molybdenum	0.02	0.02 - 300	70 – 125	85 – 110	< 25	< 8	< 50	< 16
Nickel	0.01	0.01 - 500	70 – 125	85 – 110	< 30	< 8	< 60	< 16
Phosphorus	160	160 - 20000	85 – 110	92 – 105	< 7	< 4	< 14	< 8
Potassium	150	150 - 40000	85 – 110	92 – 105	< 7	< 4	< 14	< 8
Selenium	0.01	0.01 - 10	70 – 125	80 – 115	< 30	< 13	< 60	< 26
Sodium, (NaCl)	100 Na	100 - 120000	85 – 110	95 – 102	< 8	< 3	< 16	< 6
Chloride (NaCl)	300 Cl	300 - 120000	90 – 108	95 – 102	< 7	< 3	< 14	< 6
Sulfur	50	50 - 10000	85 – 110	92 – 105	< 8	< 5	< 16	< 10
Vanadium	0.005	0.005 - 100	70 – 125	85 – 110	< 32	< 9	< 64	< 18
Zinc	0.3	0.3 - 2000	75 – 120	90 – 108	< 18	< 6	< 36	< 12

	Target Concentration, mg/kg		Accuracy, %		Repeatability, % (CV_r)		Reproducibility, % (CV_R)	
Low Priority Elements	**LOQ**	**Operational Range**	**at 2x LOQ**	**at midrange**	**at 2x LOQ**	**at midrange**	**at 2x LOQ**	**at midrange**
Antimony	7	7 - 300	80 – 115	85 – 110	< 12	< 8	< 24	< 16
Barium	10	10- 500	80 – 115	85 – 110	< 12	< 8	< 24	< 16
Bismuth	50	50 - 2000	85 – 110	90 – 108	< 8	< 6	< 16	< 12
Bromine	20	20 - 5000	80 – 115	90 – 108	< 9	< 6	< 18	< 12
Germanium	3	3 - 60	80 – 115	80 – 115	< 13	< 10	< 26	< 20
Lithium	2.5	2.5 - 50	80 – 115	80 – 115	< 13	< 10	< 26	< 20
Rubidium	0.05	0.05 - 400	70 – 125	85 – 110	< 22	< 8	< 44	< 16
Silicon	2	2 - 4000	75 – 120	90 – 108	< 13	< 6	< 26	< 12
Silver	0.3	0.3 - 200	75 – 120	85 – 110	< 18	< 8	< 36	< 16
Strontium	100	100 - 4000	85 – 110	90 – 108	< 8	< 6	< 16	< 12
Tin	10	10 - 200	80 – 115	85 – 110	< 12	< 8	< 24	< 16
Tungsten	2	2 - 40	75 – 120	80 – 115	< 13	< 12	< 26	< 24
Uranium	10	10 - 200	80 – 115	85 – 110	< 12	< 8	< 24	< 16