AGRICULTURAL MATERIALS

Practical Assay of Feed Premixes for Selenite Adsorbed on Reduced Iron

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This method assays feed premixes for added selenite in 5–10 min. It requires that selenite be added in a form easily isolated from the premix. Sodium selenite adsorbed on a reduced iron carrier serves this purpose since it can be retrieved magnetically from samples. The assay is done either indirectly by weighing the iron and calculating the amount of Se or directly by extracting the selenite from the iron carrier and determining it by titration. The indirect assay may be done any time after production of premix. The direct method requires retrieval of the additive from the premix soon after production. The actual assay may be done later. The indirect method gives high results with some matrixes unless adjusted for background ferromagnetic material. The direct method gives results with an accuracy and precision equal to those of the U.S. Food and Drug Administration's Center for Veterinary Medicine regulatory enforcement method with all matrixes studied.

The Food Drug Administration allows addition of sodium selenite to animal feeds at levels between 0.1 and 0.3 ppm. The selenite must be added as a premix, the premix must be added to the feed at no less than 1 lb/ton, and every batch of premix must be assayed for Se. For 20 years, many premix manufacturers have satisfied this requirement by using an indirect assay.

Sodium selenite adsorbed on reduced Fe (RFSe, available as Microtracer MTRFSE-2%, or 2% RFSE, and MTRFSE-4% or 4% RFSE) is a prototype product with microingredients that are magnetically retrievable and available for assay free from matrix interference. RFSe is free flowing and relatively dust-free. The Se contents should be controlled closely to conform to specifications. Premixes produced with RFSe are easily assayed for selenite.

The proposed assay uses no reagents for the indirect method and only a few common reagents for the direct method. A technician can complete 6–12 determinations per hour. The assays can be conducted in a premix-manufacturing environment. The practical procedures give timely and accurate analyses for every batch of premix.

By contrast, the U.S. Food and Drug Administration's Center for Veterinary Medicine (FDA–CVM) regulatory enforcement method developed by FDA's Denver District (1) relies on classical but potentially explosive perchloric acid digestion (2), development of a colored complex with diaminobenzidine (a suspected carcinogen), extraction of the resulting complex with toluene, and spectrophotometric determination (2–4). An experienced analyst can assay 10 samples per day, but the method requires a perchloric acid hood and glassware.

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METHOD

Apparatus

(a) *Rotary detector kit.*—Available from Microtracers, Inc., (San Francisco, CA), or equivalent. The commercial kit includes a rotary detector for retrieving at least 95% of 100–300 mesh ferromagnetic particulates from premixes. The device consists of a 2-part housing and an inner assembly of a 7 cm diameter ceramic speaker magnet mounted on the vertical shaft of a $\frac{1}{83}$ horsepower, 1550 revolution-per-minute unit bearing motor. The motor is rigidly supported by the lower member of the housing. The active face of the magnet is horizontal. Its vertical concentric poles are roughly 25 mm in diameter with a 1.25 mm gap. The entire face is covered with a 22 gauge aluminum sheet to protect the poles from contamination. A central spindle, $\frac{3}{16}$ in. (4.76 mm) in diameter, holds a circular sheet of filter paper much as a turntable holds a phonograph record. The upper part of the housing contains a funnel, the tip of which is concentric with the magnet's spindle and 5 mm above its face. Samples are introduced through the funnel, scanned by the rotating magnet, and captured in a plastic bin held in the lower housing. Iron is retained on the face of the filter paper just above the magnetic gap.

Also included in the commercial kit are a 25 mm fantail brush, an aluminum weighing scoop, a circular magnet as above but without spindle, and qualitative filter paper (7.5 cm diameter with a 4 mm hole in center).

(b) *Balance.*—Able to weigh to nearest milligram.

(c) *Sieve.*—U.S. sieve No. 6.

For the direct method, the following also are needed.

(d) *Glassware.*—Standard laboratory glassware.

(e) *Fluted filter paper.*—18.5 cm, Whatman No. 2V, or equivalent.

Reagents for Direct Method Only

(a) *Sodium thiosulfate.*—0.008N, made and standardized daily from a 0.1N stock.

(b) *Iodine.*—0.005N iodine in 2.5% KI.

(c) *HCl.*—20 mL diluted to 100 mL to make 2.5N.

(d) *Starch indicator.*—1%.

Sample Preparation

Four commercial premixes were used as matrixes. RFSe, weighed to ± 0.2 mg, was added to 25 g subsamples in pint mason jars to produce mixes of specified Se content. Each jar was capped and shaken for 2 min while being rotated and inverted. Because the entire mixture was analyzed, this procedure was a simple and accurate way to produce samples containing a known amount of analyte. For stability studies, samples with Se at 200 ppm were incubated at 35°C for various times before analysis. For calibration curves, samples with Se ranging from 0 to 400 ppm were used without incubation.

Commercial Samples

The resistance of RFSe to attrition was evaluated by examining 2 commercial premixes: a vitamin premix formulated with 8 lb of 2% RFSe per ton (80 ppm Se) and a mineral premix formulated with 80 lb of 4% RFSe per 3 tons (533 ppm Se).

Procedure

(a) *Indirect method.*—Weigh premix (ca 25 g for 200 ppm) to the nearest 0.1 g. Break up lumps and sift through a 6 mesh sieve, if necessary, before passing it through the rotary detector. Carefully remove the paper from the magnet and brush the retrieved iron into the counterpoised scoop. Make a second pass and add this retrieved iron to the scoop. Try a third pass to be sure there is no significant residual iron. If there is, add it to the scoop. If not, omit the third pass when assaying that matrix. Place the scoop on the face of the separate magnet to retain the iron. Remove extraneous matter by gentle blowing while moving the scoop about. If w is the net weight of retrieved iron (g), W is the weight of premix (g), and F is the percentage of Se in RFSe, then the amount of Se in sample (ppm) is calculated as follows:

$$\text{Se, ppm} = 10\,000 \times F \times \frac{w}{W} \qquad (1)$$

(b) *Direct method.*—Transfer the weighed iron to a 25 mL volumetric flask (or a 25 mL graduated glass-stoppered cylinder), make to volume with deionized water, and shake for 10 s to extract selenite. Filter. Prepare the solutions listed in Table 1 and titrate each with the iodine reagent.

Calculate the amount of Se in sample as follows:

Table 1. Sequence of reagent addition for the Norris-Fay titration

Reagent	Reagent blank	Sample
Sample filtrate, mL	0.00	10.00
H_2O, mL	40.00	30.00
Thiosulfate, mL	20.00	20.00
Starch indicator, drops[a]	6	6
HCl, mL[a]	2.5	2.5
Iodine titrant, mL	V_b	V_s[b]

[a] Add just before titrating.
[b] If less than 5 mL, add 5.00 mL more of thiosulfate and continue titration; adjust ($V_b - V_s$) by multiplying V_b by 25/20.

$$\text{Se, \%} = 100 \times \frac{20}{V_b} \times N \times 0.01974 \times \frac{R(V_b - V_s)}{W} \qquad (2)$$

$$= \frac{0.790}{V_b} \times \frac{V_b - V_s}{W} \qquad (3)$$

$$\text{Se, ppm} = 10\,000 \times (\text{Se,\%}) \qquad (4)$$

In equation 2, $20/V_b$ is the thiosulfate/iodine ratio determined during blank titration. When multiplied by N, the thiosulfate normality, it gives the normality of the iodine reagent. The dilution ratio, R, is the total volume of extract divided by the aliquot taken for titration, 25/10 in the procedure described. The milliequivalent weight of Se is 0.01974.

Results and Discussion

Table 2 illustrates the repeatability of the Norris-Fay titration (5) in assays of sodium selenite and RFSe.

Method precision, as measured by within-laboratory relative standard deviation, is ± 0.3%.

For all matrixes and all conditions, the reduced iron in RFSe was stable during the 15 days incubation and probably remained so during the life of the premix. Indirect assays should, therefore, be equally time-independent.

Table 2. Selenium in sodium selenite and RFSe determined by Norris-Fay titration

Sample	Selenium declared, %	n	Moisture, %	Selenium found, %	SD[a]
Sodium selenite[b]	>44.7	10	0.20	45.01	0.126
RFSe, 2%[c]	1.90–2.20	10	0.12	2.021	0.052
RFSe, 4%[d]	3.80–4.40	7	0.14	4.183	0.068

[a] Standard deviation.
[b] Spectrum lot CF 228.
[c] Microtracer lot C 2370.
[d] Microtracer lot C 2386.

In some premixes, sodium selenite appeared to migrate from the RFSe to the matrix. This movement imposed a time limit on sampling and retrieval of RFSe for the direct method. For other matrixes, possibly because of the presence of moisture or fat, retrieval of iron was less efficient. More than 2 passes through the rotary detector may be required to achieve the specified >95% recovery.

The selenite did not migrate in a limestone matrix with essentially zero moisture (Tables 3A and 3B). Recoveries were 103–121% by the indirect method and 91–102% by the direct method at incubations of up to 360 h for 2% RFSe and up to 28 h for 4% RFSe.

The vitamin premix contained 14 supplements plus rice hulls and mineral oil, each a potential interference. Recoveries were 92–102% (Tables 4A and 4B) by the indirect method. With the direct method, recoveries of 2% RFSe were 79–91% and recoveries of 4% RFSe were 74–82% during the first 4 h of incubation. These low recoveries may be due to migration of selenite from RFSE to the matrix. The possibility of fat interfering with retrieval of RFSe was minimized by passing the sample through the rotary detector 3 times.

A medicated premix with rice hulls as carrier (Tables 5A and 5B) gave recoveries of 101–112% by the indirect method over a 240 h incubation for 2% RFSe and a 28 h incubation for 4% RFSe and 94–101% by the direct method for the same incubations.

A mineral premix with 10 components and with added mineral oil (Tables 6A and 6B) yielded recoveries of 105–165% with the indirect method throughout the 24 h incubation and over a concentration range of 50–400 ppm. This premix con-

Table 3A. Stability of 2% RFSe in limestone premix incubated at 35°C

Time, h	Se added, ppm	Selenium, ppm		Selenium recovery, %	
		Indirect[a]	Direct[b]	Indirect	Direct
0	206	243	202	118	98
	202	237	206	117	102
2	214	233	207	109	97
	200	235	198	117	99
5	203	230	198	113	98
	204	232	187	114	92
19	205	237	201	116	98
	204	237	201	116	98
24	205	210	201	103	98
	202	211	196	105	97
48	203	233	200	115	99
	204	246	197	121	97
216	200	218	200	105	98
	201	208	184	103	91
360	200	208	199	104	99
	198	214	196	108	99
Average ± standard deviation				112 ± 6.1	98 ± 2.6

[a] Here and in following tables, selenium proportion of retrieved iron.
[b] Here and in following tables, Norris-Fay titration of extract from retrieved iron.

Table 3B. Stability of 4% RFSe in limestone premix incubated at 35°C

Time, h	Se added, ppm	Selenium, ppm		Selenium recovery, %	
		Indirect	Direct	Indirect	Direct
0	204	235	199	115	97
	205	242	202	118	98
2	202	226	194	112	96
	197	215	191	109	97
4	199	218	191	109	96
	210	222	198	111	98
19	206	231	196	112	95
	213	235	203	110	95
24	205	216	196	105	95
	200	204	192	102	96
28	202	223	192	110	95
	214	231	200	108	93
Average ± standard deviation				110 ± 4.2	96 ± 1.4

Table 3C. Norris-Fay titrations of aqueous extracts of retrieved 2% RFSe from limestone premix, 2% RFSe variable

Se added, ppm	Selenium, ppm		Selenium recovery, %	
	Indirect	Direct	Indirect	Direct
0.0	0.0	—	Blank	—
0.0	0.0	—	Blank	—
52.2	48.9	43.6	93.7	88.7
50.7	51.6	47.3	101.7	93.3
99.3	96.4	95.1	97.1	95.8
96.8	98.7	96.2	102.0	99.4
204.8	193.0	181.8	94.2	88.8
207.2	194.1	182.3	93.7	88.1
300.4	282.6	249.9	94.1	83.2
301.0	294.9	270.2	98.0	89.8
401.0	390.7	358.6	97.4	89.5
408.9	410.3	361.4	100.3	88.2
Average ± standard deviation			97 ± 3.2	90 ± 4.6

Table 4A. Stability of 2% RFSe in vitamin premix incubated at 35°C

Time, h	Se added, ppm	Selenium, ppm		Selenium recovery, %	
		Indirect	Direct	Indirect	Direct
0	199	194	176	98	88
	199	193	173	97	87
2	206	210	188	102	91
	202	205	179	102	89
4	206	199	164	96	79
	200	201	164	101	82
24	205	195	138	95	67[a]
	208	211	153	101	73[a]
Average ± standard deviation				99 ± 2.8	86 ± 4.6

[a] Low recoveries resulting from selenite migration, more pronounced with increasing time, were omitted from average.

tained background ferromagnetic material and gave false high values with the indirect method. A background correction can be made by deducting a blank, the amount of ferromagnetic material in the premix prior to addition of RFSe. The blank can also be estimated as the difference between the indirect and direct assays, particularly at low addition levels. In Table 6B, for Se added at 50 ppm, by subtracting data in the third column from those in the second column, we find blanks of 83.0 – 47.3 = 35.7 and 86.8 – 48.3 = 38.5 ppm, averaging 37.1 ppm. With this value, recoveries for the entire range, 50–400 ppm, become 90–108%. The direct method gave recoveries of 83–98%.

Calibration data are reported in Tables 3C, 4C, 5C, and 6B. Data are summarized in Tables 7 and 8. Table 9 demonstrates the linearity of both indirect and direct methods. The regression

Table 4B. Stability of 4% RFSe in a vitamin premix incubated at 35°C

Time, h	Se, ppm added	Selenium, ppm		Selenium recovery, %	
		Indirect	Direct	Indirect	Direct
0	211	194	164	92	78
	209	195	169	93	81
2	201	193	158	97	79
	213	206	175	97	82
4	198	193	156	97	79
	206	196	153	95	74
24	213	204	123	96	58[a]
	201	187	105	93	52[a]
Average ± standard deviation				95 ± 2.1	79 ± 2.8

[a] Low recoveries result from selenite migration, more pronounced with increasing time. They are omitted from average.

Table 4C. Norris-Fay titrations of aqueous extracts of retrieved 2% RFSe from vitamin premix, 2% RFSe variable

Se added, ppm	Selenium, ppm		Selenium recovery, %		
	Indirect	Direct	Indirect	Indirect, net	Direct
0.0	0.6	—	Blank	—	—
0.0	0.9	—	Blank	—	—
53.2	53.2	45.7	100.0	98.6	85.9
52.1	55.1	47.6	105.7	104.3	91.4
101.4	102.8	86.6	101.5	100.6	85.5
98.9	98.8	84.7	99.9	99.1	85.6
199.4	194.3	175.9	97.5	97.1	88.3
198.5	192.7	173.2	97.1	96.7	87.3
302.4	301.8	274.4	99.8	99.6	90.7
302.5	299.8	247.6	99.1	98.9	81.9
405.6	412.5	340.2	101.7	101.5	83.9
395.1	398.7	327.8	100.9	100.7	83.0
Average			100.3	99.7	86.4
Standard deviation			± 2.4	± 2.2	± 3.1

intercept differed significantly from zero only for the mineral premix analyzed by the indirect method, reflecting the presence of extraneous ferromagnetic material. The slopes measure recoveries ranging from 84 to and 110% for both indirect and direct methods. Standard errors of the estimate for Se vary between ± 2.6 and ± 7.2 ppm for the 4 matrixes.

Recoveries and coefficients of variation (CVs) for data from indirect assay are summarized in Table 7. Recoveries vary from

Table 5A. Stability of 2% RFSe in medicated premix incubated at 35°C

Time, h	Se added, ppm	Selenium, ppm		Selenium recovery, %	
		Indirect	Direct	Indirect	Direct
0	206	208	206	101	100
	200	204	201	102	101
2	201	202	201	101	100
	197	202	196	103	100
5	195	202	191	104	98
	202	205	201	102	100
24	206	213	201	103	97
	200	204	199	102	100
240	201	211	203	105	101
	203	211	199	104	98
Average ± standard deviation				103 ± 1.3	100 ± 1.4

Table 5B. Stability of 4% RFSe in a medicated premix incubated at 35°C

Time, hrs	Se added, ppm	Selenium, ppm		Selenium recovery, %	
		Indirect	Direct	Indirect	Direct
0	206	221	199	107	96
	206	217	203	105	99
2	202	211	201	105	100
	212	227	207	107	98
4	212	237	201	112	95
	209	226	201	108	96
19	201	215	193	107	96
	210	218	203	109	97
24	215	231	202	107	94
	206	228	200	111	97
28	207	226	202	109	98
	206	225	200	109	97
Average ± standard deviation				108 ± 2.1	96 ± 2.6

92 to 121% (only background-adjusted data for the mineral premix were considered). CVs range between ± 0.8 and ± 2.9%. Two commercial premixes made with RFSe were assayed for Se to evaluate resistance of RFSe to mechanical abuse. The vitamin premix made with 2% RFSe was 2 days old

Table 5C. Norris-Fay titrations of aqueous extracts of retrieved 2% RFSe from medicated premix, 2% RFSe variable

Se added, ppm	Selenium, ppm		Selenium recovery, %		
	Indirect	Direct	Indirect	Indirect, net	Direct
0.0	1.4	—	Blank	—	—
0.0	1.7	—	Blank	—	—
50.4	52.9	50.0	104.9	101.2	99.2
49.9	51.4	49.1	103.1	101.7	98.4
100.1	103.5	96.9	103.4	101.9	96.7
99.9	103.1	99.6	103.2	101.6	99.8
207.9	209.9	194.3	100.9	100.2	93.5
202.5	206.0	196.3	101.7	101.0	97.0
299.9	296.4	272.0	98.8	98.3	90.7
295.1	297.1	272.9	100.7	100.2	92.5
410.3	409.5	396.1	99.8	99.4	96.6
399.4	406.4	391.9	101.8	101.4	98.2
Average			101.8	100.7	96.3
Standard deviation			± 1.9	± 1.2	± 3.0

Table 6A. Stability of 4% RFSe in mineral premix incubated at 35°C

Time, h	Se added, ppm	Selenium, ppm		Selenium recovery, %	
		Indirect	Direct	Indirect	Direct
0	211	259	192	123	91
	200	210	165	105	83
2	209	251	186	120	89
	213	235	186	111	87
4	207	255	199	123	96
	204	247	189	121	93
24	210	283	205	135	98
	211	268	206	127	98
Average ± standard deviation				121 ± 9.2	92 ± 5.4

when assayed. The mineral premix made with 4% RFSe was 12 days old. Se in the vitamin premix was 103% of the specified Se content as determined by the indirect method and 83% by the direct method. This low recovery by the direct method was probably due to the age of the sample. The mineral premix contained much extraneous ferromagnetic material as expected, and the direct method was used. Fifteen samples taken from one batch were each assayed to evaluate the uniformity of the mix. The Se found was 87% of the value specified, and the CV was ± 3.3%.

Table 6B. Norris-Fay titrations of aqueous extracts of retrieved 2% RFSe from mineral premix, 2% RFSe variable

Se added, ppm	Selenium, ppm		Selenium recovery, %		
	Indirect	Direct	Indirect	Indirect, net	Direct
0.0	23.6	0.0	Blank	—	—
0.0	26.5	0.0	Blank	—	—
50.9	83.0	47.3	163.6	113.8	92.9
52.6	86.8	48.3	165.2	117.3	91.8
101.1	132.7	94.1	132.3	106.4	93.1
96.5	132.0	94.5	136.7	110.8	97.9
199.2	238.2	192.8	119.6	107.0	96.8
201.2	234.3	192.2	116.5	104.0	95.5
303.4	355.2	292.3	117.0	108.8	96.3
298.1	356.2	274.9	119.5	102.8	92.2
405.4	466.9	374.1	115.2	109.0	92.3
405.4	476.5	376.1	111.4	111.3	92.8
Average			129.7	109.1	94.2
Standard deviation			± 19.9	± 4.4	± 2.2

Table 7. Summary of recoveries by indirect method, Se added at 200 ppm unless otherwise indicated

Table	n	Incubation, h	Recovery, %	Pooled CV, %
		Limestone		
3A	16	0–360	103–121	1.9
3B	12	0–28	102–118	1.6
3C[a]	10	0	94–102	2.9
		Vitamin premix		
4A	8	0–24	93–102	2.2
4B	8	0–24	92–97	1.1
4C[a]	10	0	97–106	2.7
		Medicated premix		
5A	10	0–240	101–105	1.0
5B	12	0–28	105–112	1.5
5C[a]	10	0	99–105	0.8
		Mineral premix		
6A	8	0–24	105–135	5.5
6B[a]	10	0	111–165	1.8
6B[a] less background	10	0	104–117	2.5

[a] Selenium varied from 0 to 400 ppm.

Table 8. Summary of recoveries by direct method, Se added at 200 ppm unless otherwise indicated

Table	n	Incubation, h	Recovery, %	Pooled CV, %
		Limestone		
3A	16	0–360	91–102	3.0
3B	12	0–28	93–98	0.9
3C[a]	10	0	83–99	2.7
		Vitamin premix		
4A	6	0–4	79–91	1.7
4B	6	0–4	74–82	3.3
4C[a]	10	0	83–91	2.7
		Medicated premix		
5A	10	0–240	97–101	1.3
5B	12	0–28	94–100	1.3
5C[a]	10	0	91–99	1.6
		Mineral premix		
6A	8	0–24	83–98	2.6
6B[a]	10	0	92–98	1.8

[a] Selenium varied from 0 to 400 ppm.

Table 9. Linear regressions derived from calibration data[a] (Se, ppm)$_{calc}$ = b_0 + b_1(Se, ppm)$_{present}$, n = 12

Matrix/table	b_0	$b_1 \pm SE^b$	r^{2c}	SE of est.
Limestone, 3C				
Indirect	−1.235	0.987 ± 0.012	0.998	± 6.1
Direct	2.266	0.876 ± 0.013	0.998	± 6.6
Vitamin, 4C				
Indirect	−0.545	1.004 ± 0.008	0.999	± 3.7
Direct	2.477	0.841 ± 0.015	0.997	± 7.2
Medicated, 5C				
Indirect	2.125	0.999 ± 0.005	1.000	± 2.6
Direct	0.177	0.954 ± 0.014	0.998	± 6.8
Mineral, 6B				
Indirect	24.40	1.097 ± 0.011	0.999	± 5.2
Direct	1.642	0.931 ± 0.009	0.999	± 4.3

[a] Derived from data in Tables 3C, 4C, 5C, and 6B.
[b] SE, the standard error of the estimate.
[c] r^2, the correlation coefficient squared (coefficient of determination).

Recoveries with the direct method for all matrixes and conditions ranged from 74 to 102%, and CVs ranged from 0.9 to 3.3% (Table 8). These results compare favorably with those obtained by 2 more arduous procedures being proposed for regulatory monitoring of Se premixes (FDA–CVM method): using Procedure 1 applicable only to mineral premixes, recoveries in a 4-laboratory trial ranged from 82 to 101%, with CVs ranging from 0.8 to 9.9%. Using Procedure 2 applicable to feed-based premixes, recoveries in a single laboratory study ranged from 76.5 to 104%, with CVs ranging from 0.2 to 9.4%.

Conclusions

The direct method permits practical, accurate, and timely in-plant Se assays of selenite-containing premixes produced with sodium selenite adsorbed on reduced iron. It compares in accuracy with methods proposed by FDA–CVM. The indirect method may give false high recoveries in matrixes containing extraneous ferromagnetic material unless appropriate blanks are deducted.

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