Application Note

Food Testing
and Agriculture

High Throughput Analysis of Animal Feed using the Agilent 7800 ICP-MS with ISIS 3

Surpassing EN 17053:2018 Standard Method requirements for multiple elements in animal feed

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Introduction

The quality of feed given to livestock has a direct impact on the animal's health and welfare. It also affects the dietary intake of anyone who consumes animal-derived produce. Monitoring the elemental content of animal feed provides farmers with information on nutrients and additives, so they can choose the most suitable feed for their livestock. It also ensures food safety by identifying residues or contaminants. The European Union has recently released a new European Standard Method, EN 17053:2018 "Animal feeding stuffs - Methods of sampling and analysis - Determination of trace elements, heavy metals, and other elements in feed by ICP-MS (multi-method)" (*1*). The standard includes 13 elements: As, Cd, Co, Cu, Fe, Hg, Mn, Mo, Pb, Se, Tl, U, and Zn. In routine analysis however, manufacturing quality assurance and quality control (QA/QC) laboratories typically determine nutrient elements (Ca, K, Na, Mg, P, S), other minor elements (Ni, Sr, Al, B, Cr, and V).

There are a large variety of feeds, reflecting different animals' requirements, and a range of feed types such as pellets, grasses/hays, and grains. Feed is produced in large quantities, so a high number of test-samples are generated. ICP-MS is suited to the quantitative analysis of feeds because of its multi-element capability, high sensitivity, and speed. The analysis can be challenging though because high concentrations of major nutrient elements including Ca, K, Mg, and Na contribute to the total dissolved solid (TDS) content of samples. High matrix samples can affect instrument stability and accuracy. The large number of elements contained within animal feeds can lead to the formation of various polyatomic interferences that affect certain elements. Also, the wide concentration range of elements in feeds causes significant variance in matrix levels that may require more than one dilution for each sample to satisfy the analysis requirements.

The Agilent 7800 ICP-MS fitted with the ISIS 3 and SPS 4 autosampler addresses these analytical concerns, making it suitable for the routine analysis of animal feed:

1. The 7800 uses a high-temperature, robust plasma, as indicated by a low CeO/Ce ratio of <1%, which effectively decomposes many sample matrices. For high matrix samples with a TDS content up to 3%, the High Matrix Introduction (HMI), which is standard on the 7800, uses aerosol dilution to reduce matrix loading on the interface. HMI also simplifies sample preparation by reducing the time needed and potential errors associated with additional liquid dilution (*2*). HMI mode is accessed via one of the preset methods in the ICP-MS MassHunter software, simplifying method development for routine analysis. The ICP-MS MassHunter software also features auto-optimization and auto-start up, making it easier to develop reliable methods.
2. The 7800 ICP-MS provides effective interference removal with its fourth-generation Octopole Reaction System (ORS^4) collision/reaction cell (CRC). The ORS^4 is optimized for helium collision mode (He mode) which uses kinetic energy discrimination (KED) to reduce the transmission of all common matrix-based polyatomic interferences (*3*). High Energy helium mode (HEHe) can be used to break up polyatomic ions with a higher bond energy, without the need to use a reactive gas. Avoiding reactive gases, such as H_2 or NH_3, using He mode ensures that no new molecular interferences are formed in the CRC, improving the quality of the data.
3. The 7800 ICP-MS is fitted with an Orthogonal Detector System (ODS), which ensures high sensitivity and a wide dynamic range with 10 orders of magnitude. The detector allows trace elements (<1 ppt), minor (ppb), and major nutrients (100s or 1000s of ppm) to be measured in the same run. The high upper concentration limit reduces sample reruns caused by over range results, allowing labs to maintain high productivity when analyzing variable sample matrices.
4. Agilent's third-generation Integrated Sample Introduction System (ISIS 3) discrete sampling device is optimized for high sample throughput. The ISIS 3 uses a high-speed piston pump to rapidly fill (load) the sample loop. A discrete volume of the sample is then injected into a flowing carrier stream using a 7-port switching valve. Meanwhile the autosampler probe and autosampler line are thoroughly washed in preparation for analysis of the next sample. ISIS 3 speeds up analysis times by separating the sample uptake and rinse steps from the acquisition step (*4*).

In this application, 25 elements, including the 13 listed in the EN17053:2018 standard, were determined in animal feed. Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Sr, Tl, U, V, and Zn, were analyzed by the Agilent 7800 ICP-MS fitted with the ISIS 3 and SPS 4 autosampler.

Instrumentation

An Agilent 7800 ICP-MS fitted with the standard HMI, Ni interface cones, ORS^4, and the optional ISIS-3 was used. The ICP-MS was configured with the standard sample introduction system consisting of a MicroMist glass concentric nebulizer, quartz Scott-type spray chamber, and quartz torch with a 2.5 mm id injector. An Agilent SPS 4 autosampler was used to introduce the standards and samples to the ICP-MS.

For easy method development and fast start-up, instrument conditions were set using the "High Matrix" preset method. By selecting "HMI-4" in the ICP-MS MassHunter software, the plasma conditions listed in Table 1 (shaded) were set automatically. ISIS 3 parameters are listed in Table 2. The average sample-to-sample analysis time for the measurement of 25 analytes was 122 seconds.

Table 1. ICP-MS operating conditions.

ICP-MS parameter	He mode
RF power (W)	1600
Sample depth (mm)	10
Nebulizer gas flow rate (L/min)	0.62
Dilution gas flow rate (L/min)	0.37
Spray chamber temperature (°C)	2
Pump speed (rps)	0.10
Lens tune	Autotune
He Cell gas flow rate (mL/min)	5.0 (9.0*)
Energy discrimination (V)	5.0 (7.0*)
Data acquisition time (s)	77

HMI conditions (shaded parameters) were automatically optimized during startup.
**HEHe mode settings used for S, P, and Se.*

Table 2. ISIS 3 settings.

Parameter	Setting	
Loop length (2 mm i.d.) (cm)	25	
Loop volume (mL)	0.80	
	Time uptake (s)	Pump speed (%)
Sample load (s)	7	36
Stabilization (s)	20	5
Probe rinse (sample) (s)	25	5
Probe rinse (standard) (s)	25	5
Probe rinse 1 (s)	5	80
Probe rinse 2 (s)	10	5
Optional loop probe wash (s)	5	60
Optional loop wash (s)	9	36
Sample-to-sample analysis time (s)	122	

Standard preparation

The calibration standards and QC standards were prepared using a multi-elemental environmental calibration standard (Agilent Technologies, p/n 5183-4688), 1000 mg/L single elemental standards (Al, B, Hg, Sr), and 10,000 mg/L calibration standards (Ca, K, P, S) (Kanto Chemical Co., Inc, Japan). The concentration ranges are given in Table 3. The single elemental standards for Al, Ca, and K were used to extend the calibration range for these major elements. Electronic grade (EL) nitric acid and hydrochloric acid (Kanto Chemical Co., Inc, Japan) were used to matrix match the calibration standards to the samples.

Table 3. Concentration of calibration standards.

Elements	Concentration range (ppb)
Hg	0.2 to 2
B	0.2 to 200
Al, Sr	2 to 2000
As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Tl, U, V, Zn	0.1 to 1000
Fe, Mg, Na, P, S	10 to 100,000
K	10 to 200,000
Ca	10 to 500,000

A Continuing Calibration Verification (CCV) standard was prepared with a concentration at the halfway point of the calibration curve. The CCV standard was measured periodically every 12 samples.

An internal standard (ISTD) solution containing 1 ppm ^{6}Li, Sc, Ge, Y, In, Tb, Bi, and Rh was prepared from Agilent Internal Standard mix (p/n 5183-4681) plus rhodium (Kanto Chemical, Co., Inc, Japan). The solution was automatically added to the carrier/sample flow using the ISIS 3, giving it an approximate dilution of 1:15. Only Li, Sc, Y, Rh, and Tb were used for the analysis.

Sample preparation

A variety of sample-types was selected to represent samples that are typically analyzed in feed-testing laboratories. Three certified reference materials (CRMs) were used including ERM-CD281 Rye Grass (IRMM, Belgium), IPE-133 Maize and IPE-148 Lucerne (WEPAL, The Netherlands). Two animal feeds, a chicken grain mix and rabbit grass, were bought in a local store. Both feed samples were chopped and milled to a particle size of less than 0.5 mm.

The CRMs and samples were prepared using a single microwave digestion method and diluted only once. A subsample of 0.20 g was accurately weighed and placed in a PTFE microwave vessel and 1.5 mL of HNO_3 and 0.75 mL of HCl was added. The samples were left for 30 minutes before being placed into a microwave (Mars 6, CEM) and digested using the program given in Table 4. The fully digested samples were then diluted to 50 mL with de-ionized water.

Table 4. Microwave digestion parameters.

Stage	Temperature (°C)	Time (min)	Power (W)
1 - ramp	180	5	600
2 - ramp	210	10	1800
3 - hold	210	30	1800
4 - cool	-	30	-

Results and discussion

Calibration

Calibration coefficients exceeding 0.9999 were achieved for all elements, demonstrating the ability of the 7800 ICP-MS to measure analytes across a wide-concentration range. As shown by the four representative calibration curves shown in Figure 1, excellent linearity was achieved for major elements (Ca, up to 500 ppm) and trace elements (As and Cd, up to 1000 ppb, and Hg to 2 ppb).

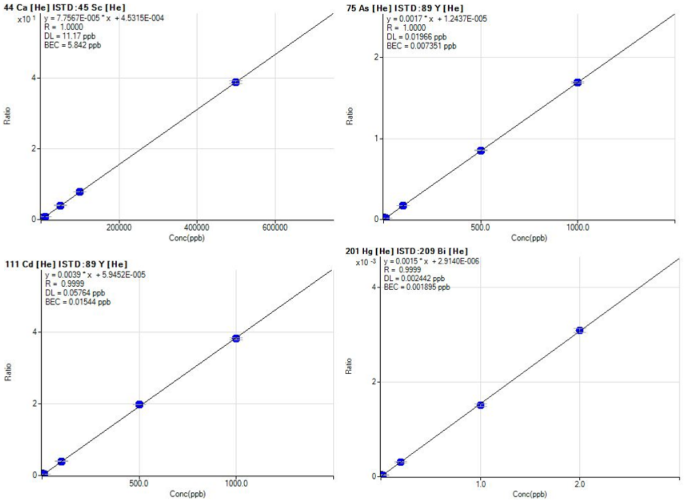

Figure 1. Linear calibration curves for ^{44}Ca, ^{75}As, ^{111}Cd, and ^{201}Hg.

All analytes were measured in He mode using the acquisition parameters listed in Table 5. Three sigma method detection limits (MDLs) and 10 sigma Limit of Quantitation (LOQs) were calculated from 10 measurements of the matrix-matched calibration blank (Table 5). The reported LOQs (corrected for dilution) were significantly below the specifications in the EN 17053:2018 standard, confirming the sensitivity of the 7800 ICP-MS for the application.

Table 5. 7800 ICP-MS acquisition parameters, MDLs, LOQs in animal feed samples, and LOQs required by the EN 17053:2018 standard method.

Element and mass number	Integration time (s)	MDL in solution (ppb)	LOQ in animal feed (mg/kg)	LOQ required by EN:17053 (mg/kg)
11 B	0.3	0.81	0.66	
23 Na	0.3	2.49	2.05	
24 Mg	0.3	0.51	0.42	
27 Al	0.5	0.66	0.54	
31 P	0.3	2.21	1.82	
34 S	0.3	215	177	
39 K	0.3	10.4	8.55	
44 Ca	0.3	5.82	4.80	
51 V	0.3	0.011	0.009	
52 Cr	0.3	0.023	0.019	
55 Mn	0.3	0.033	0.027	0.10
56 Fe	0.3	0.18	0.15	5.0
59 Co	0.3	0.004	0.003	0.10
60 Ni	0.3	0.053	0.04	
63 Cu	0.3	0.034	0.028	
66 Zn	0.5	0.907*	0.75	5.0
75 As	2.0	0.012	0.010	0.05
78 Se	3.0	0.040	0.033	
88 Sr	0.3	0.019	0.016	
95 Mo	0.3	0.005	0.004	0.10
111 Cd	0.3	0.010	0.008	0.03
201 Hg	1.0	0.003	0.002	0.04
205 Tl	0.3	0.007	0.006	0.10
208 Pb**	0.6 (3 x 0.2 s)	0.006	0.005	0.10
238 U	0.1	0.001	0.001	0.10

**The instrument DL of Zn was 0.12 ppb, suggesting contamination of Zn from the lab environment.*

***Pb is measured as the sum of the three most abundant isotopes, 206, 207, 208.*

ISTD recoveries

For the ISTD recovery test, 266 solutions were analyzed and ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, and ^{159}Tb were monitored over nine hours. As shown in Figure 2, all ISTD measurements were within the ±20% control limit. The excellent stability results demonstrate the high matrix tolerance of the 7800 ICP-MS with HMI over long runs, without the need to recalibrate. Also, the robust plasma conditions were able to decompose the variable sample matrices effectively, with no significant matrix deposition on the interface during the sequence. Instrument stability is an important factor in the productivity performance of laboratories with a high sample workload.

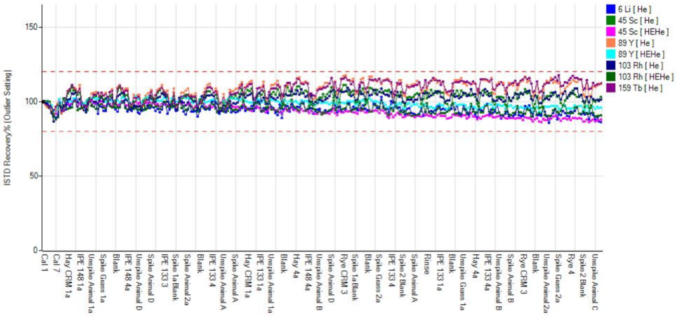

Figure 2. ISTD recoveries for 266 solutions over nine hours.

QC recovery

The CCV standards were measured periodically every 12 samples. While no method requirements for CCV recovery are given in the EN 17053:2018 standard, ±10% is a widely accepted QC recovery limit. If a QC recovery of an analyte falls outside these limits, a recalibration is often triggered. Figure 3 shows the QC recovery of the CCV standards over nine hours to be within the ±10% limit.

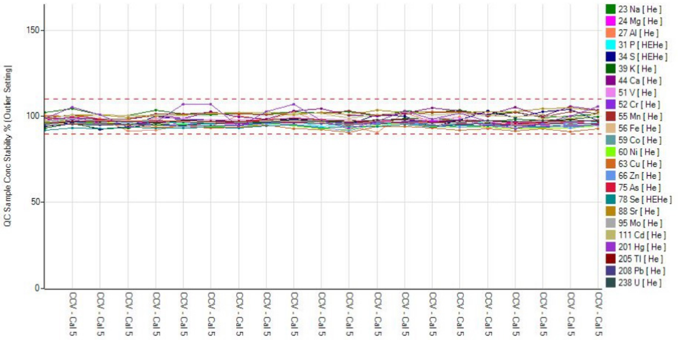

Figure 3. QC recovery of CCV standards over nine hours.

CRM recovery

The three CRMs were prepared in duplicate and measured in triplicate using one instrument, carried out on separate days. Table 6 shows the mean measured concentrations in the CRMs, and the mean recoveries compared to the certified values. The recovery results for nearly all certified elements were ±10% of the value listed on the certificates. The data demonstrates the accuracy of the 7800 ICP-MS and the effectiveness of interference removal using the ORS[4] operating in He mode. The recovery for ^{23}Na in IPE 148 Lucerne sample of 86% can be explained by the large uncertainty of the certified value. The recovery for ^{31}P in CD281 Rye Grass was ±15% of the reference value. The recovery data was acquired using the same analytical method and the same level of dilution for all elements. The results for 3.3% of K and 5 µg/kg of Hg in CD281 and IPE 133, respectively, show that good accuracy was achieved over a wide dynamic range.

Spike recoveries

Certified values were not available for all elements analyzed in the method, so a spike recovery test was performed to validate the performance of the 7800 ICP-MS. Chicken grain mix and rabbit grass samples were prepared using the same single microwave digestion method as used for the CRMs, with a final dilution factor of 250x. The spike recoveries for all elements were ±10% of the expected values, as shown in Table 7. The ODS detector, with its 10 orders of dynamic range, successfully measured elements such as Ca at ~300,000 ppb (300 ppm) to Tl at 0.009 ppb (9 ppt) in the same analytical run.

Table 6. The recovery of three certified reference materials (CRMs) for all elements. n=6. Italics indicate reference values. Dashes indicate no certified or reference value.

	EDM-CD281 Rye Grass				IPE 133 Maize				IPE 148 Lucerne			
	Measured concentration (mg/kg)	Certified concentration (mg/kg)	Uncertainty (mg/kg)	Recovery (%)	Measured concentration (mg/kg)	Certified concentration (mg/kg)	Uncertainty (mg/kg)	Recovery (%)	Measured concentration (mg/kg)	Certified concentration (mg/kg)	Uncertainty (mg/kg)	Recovery (%)
11 B	5.28	5.50	0.5	96	2.82	-			33.93	34.4	2.69	99
23 Na	3661	*4000*		92	94.5	103	22.5	92	173	200	35.3	86
24 Mg	1485	*1600*		93	1129	1150	85	98	1583	1640	102	97
27 Al	30.5	-			98.9	-			348	-		
31 P	2416	*2800*		86	1389	1490	105	93	2234	2330	118	96
34 S	3173	*3400*		93	861	935	86.3	92	2833	2950	207	96
39 K	33089	*34000*		97	7886	7890	465	100	25739	26200	1330	98
44 Ca	6167	*6300*		98	1749	1870	151	94	21358	22800	1500	94
51 V	0.22	-			0.39	0.40	0.04	98	0.85	0.810	0.15	105
52 Cr	23.8	24.8	1.3	96	0.41	0.45	0.11	91	0.858	0.886	0.17	97
55 Mn	82.0	82.0	4	100	124	122	8.1	102	40.6	40.1	2.78	101
56 Fe	175	*180*		97	194	191	19	102	247	233	27.7	106
59 Co	0.24	-			0.08	0.08	0.01	102	0.29	0.28	0.06	104
60 Ni	15.0	15.2	0.6	99	0.54	0.52	0.07	104	1.11	1.07	0.12	104
63 Cu	10.5	10.2	0.5	103	6.40	6.51	0.59	98	4.05	4.32	0.54	94
66 Zn	31.4	30.5	1.1	103	48.3	47.6	3.58	101	19.5	19.9	1.74	98
75 As	0.040	0.042	0.01	95	0.15	0.14	0.02	104	0.43	0.43	0.05	100
78 Se	0.021	0.023	0.004	93	0.02	-			0.13	0.12	0.03	107
88 Sr	22.4	-			3.15	3.31	0.28	95	53.6	55.5	3.2	97
95 Mo	2.06	2.22	0.12	93	0.16	0.17	0.03	96	0.35	0.36	0.05	96
111 Cd	0.12	0.12	0.007	98	0.27	0.26	0.02	105	0.050	0.051	0.01	98
201 Hg	0.016	0.016	0.0022	102	0.0056	0.0054	0.001	103	0.0086	0.0083	0.0007	104
205 Tl	0.01	-			0.008				0.02	-		
208 Pb	1.70	1.67	0.11	102	0.90	0.92	0.09	97	0.86	0.86	0.07	100
238 U	0.007	-			0.01				0.04	-		

Table 7. Spike recoveries of chicken grain and rabbit feed.

Element and mass	Spike concentration (ppb)	Unspiked chicken grain (ppb)	Spiked chicken grain (ppb)	Spike recovery (%)	Unspiked rabbit grass (ppb)	Spiked rabbit grass (ppb)	Spike recovery (%)
11 B	50	33.9	86.1	104	13.6	65.0	103
23 Na	10000	4548	14952	104	118	10590	105
24 Mg	4000	7670	12014	109	3740	7872	103
27 Al	300	181	488	102	42.3	351	103
31 P	5000	16916	22114	104	6834	12245	108
34 S	8000	9219	17502	104	4377	12809	105
39 K	20000	21373	41758	102	67297	86456	96
44 Ca	20000	301163	322403	106	7098	26937	99
51 V	10	0.40	10.6	102	0.15	10.4	103
52 Cr	50	0.96	51.9	102	0.086	50.8	102
55 Mn	200	320	526	103	110	318	104
56 Fe	500	312	837	105	110	648	108
59 Co	1	0.34	1.23	90	0.089	1.02	93
60 Ni	100	4.64	103	99	1.94	105	103
63 Cu	50	42.8	97.4	109	13.6	68.6	110
66 Zn	100	200	303	103	154	263	109
75 As	1	0.39	1.29	91	0.10	1.04	94
78 Se	1	0.84	1.78	94	0.015	0.98	97
88 Sr	50	1025	1074	98	30.2	81.3	102
95 Mo	10	2.28	13.0	107	1.20	11.6	104
111 Cd	1	0.52	1.46	93	0.059	1.01	95
201 Hg	1	0.014	1.01	100	0.018	1.01	99
205 Tl	10	0.13	10.3	102	0.009	10.2	102
208 Pb	10	0.30	10.7	104	0.050	10.7	107
238 U	10	0.078	10.1	100	0.023	10.2	101

Conclusion

A variety of animal feed CRMs and sample digests were analyzed using the Agilent 7800 ICP-MS with HMI and ISIS 3 discrete sampling device. The method was optimized for high throughput by optimizing the sample analysis at 122 s. ISIS 3 significantly reduces the sample uptake and rinse times and eliminates the need for stabilization after high-speed uptake, ensuring quick analysis times.

A "High Matrix" preset method in ICP-MS MassHunter was used to simplify the development and operation of the method. The 7800's robust plasma and HMI allowed the complex and highly variable animal feed samples to be measured using a single sample preparation method. Once the samples had been prepared using microwave digestion, no further liquid dilution was necessary. HMI aerosol dilution replaces conventional liquid dilution, eliminating the time and cost associated with a typical manual or automated dilution step.

The accuracy of the method was evaluated by analyzing three CRMs and spiking two real animal feed samples. Excellent recoveries were achieved for all elements across a wide concentration range using He mode to remove all common polyatomic interferences. The 7800 ICP-MS exceeded detection limit requirements specified in the EN 17053:2018 standard method and showed excellent stability over a nine-hour ISTD recovery test and a QC recovery run.

The method is suitable for the routine, multi-element analysis of animal feeds according to standard methods, such as EN 17053:2018. It is also suitable for QA/QC testing of nutrient elements and other minor and trace elements.

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