APPLICATIONS

Determination of Sugars in Animal Feed using HPLC-ELSD

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Scientist- Phenologix Outside of the lab, Laura enjoys spoiling her dog Maggie and subjecting her husband to novel methods of torture, such as endless playlists of sad songs and long walks on the beach to catch Pokémon.

Introduction

Animal feed can contain sugars from a variety of sources including plant material and binders such as molasses. Sugar content can vary widely depending on the type/maturity of grains used and the amount/type of molasses added. Even comparing a single type of grain, the sugar content can be greatly affected by growing and harvesting conditions. Because of this variability, sugar content can be difficult to predict, and testing is often required to ensure nutritional and dietary requirements are met.

In this work, a HPLC-ELSD method was evaluated for the analysis of six free nutritional sugars in animal feed: fructose, galactose, glucose, sucrose, maltose, and lactose. Assay performance including precision and recovery were evaluated and met the acceptance criteria. A Luna® Omega SUGAR LC Column was selected for its ability to retain polar sugar molecules and differentiate individual sugars using HILIC conditions. The HPLC methodology allowed for a simple sample extraction with ethanol and water followed by filtration.

Materials and Methods

Reagents and chemicals were obtained from Sigma-Aldrich® (St. Louis, MO).Two animal feed samples were obtained from a local feed store; one grain feed (corn, oats, and barley mix) and one alfalfa feed (Lomita, CA).

Sample Extraction and Clean up

1. Finely grind animal feed with spice grinder or similar.
2. Weigh out 1 g of ground sample into a centrifuge tube.
3. Add 25 mL of 50:50 ethanol/water to the tube.
4. Shake the tube on a shaker for 1 hour at room temperature.
5. Bring volume up to 50 mL with 50:50 ethanol/water and invert to mix well.
6. Centrifuge samples for 15 minutes at max rpm.
7. Filter supernatants with 0.45 µm PTFE syringe filters (Phenex™-PTFE 4 mm Syringe Filters, P/N AF0-3102-12)

Sample Preparation

Reagents and chemicals were obtained from Sigma-Aldrich® (St. Louis, MO).Two animal feed samples were obtained from a local feed store; one grain feed (corn, oats, and barley mix) and one alfalfa feed (Lomita, CA).

Calibrator Level	Final Conc. (mg/mL)	Spiking Solution	Spiking Solution Volume (µL)	Diluent or Matrix Extract (µL)
Calibrator 6	3.0	100 mg/mL Stock Standard	30	970
Calibrator 5	2.5	100 mg/mL Stock Standard	25	975
Calibrator 4	2.0	100 mg/mL Stock Standard	20	980
Calibrator 3	1.5	100 mg/mL Stock Standard	15	985
Calibrator 2	1.0	100 mg/mL Stock Standard	10	990
Calibrator 1	0.5	Calibrator 2	250	250

LC Conditions

Column: Luna Omega 3 µm SUGAR
Dimension: 150 x 4.6 mm
Part No.: 00F-4775-E0
Mobile Phase: Acetonitrile/Water (75:25) (premixed)
Flow Rate: 1.0 mL/min
Inj. Vol.: 10 µL
Col. Temp.: 35 °C
Drift Tube Temp: 45 °C
Detection: ELSD
Sample: 1. Fructose (3.9773 min)
2. Glucose (4.417 min)
3. Galactose (4.7043 min)
4. Sucrose (5.8790 min)
5. Maltose (6.8300 min)
6. Lactose (7.8235 min

Figure1.
Calibrator 1 (0.5 mg/mL)

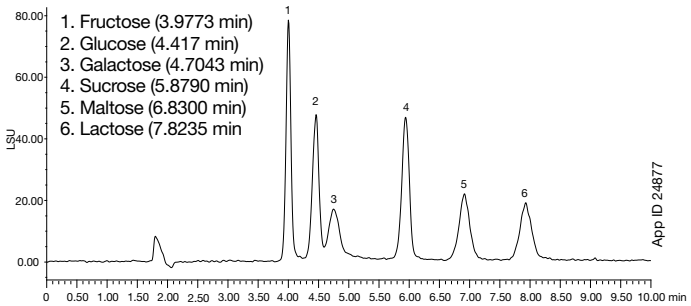

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Figure2.
Calibrator 6 (3 mg/mL)

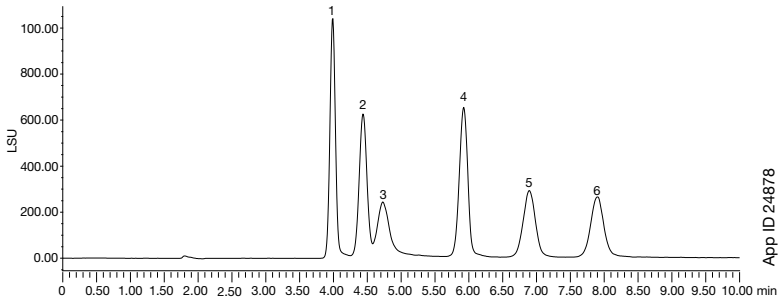


Figure 3.
Grain Feed (Unspiked)

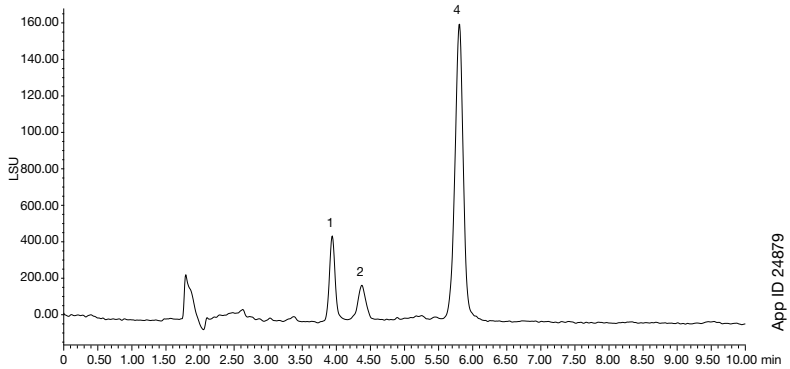


Figure 4.
Spiked Grain Feed (1mg/mL added)

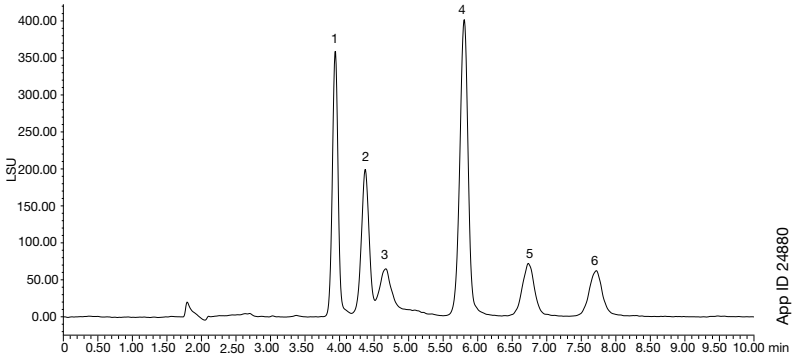
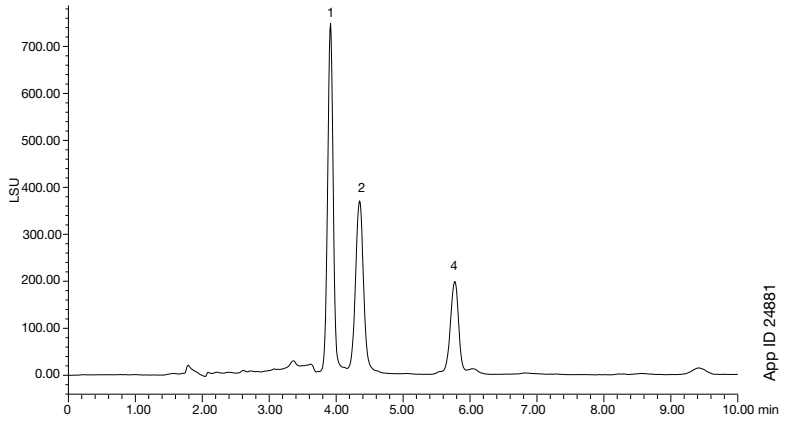


Figure 5.
Alfalfa Feed (Unspiked)



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Fructose

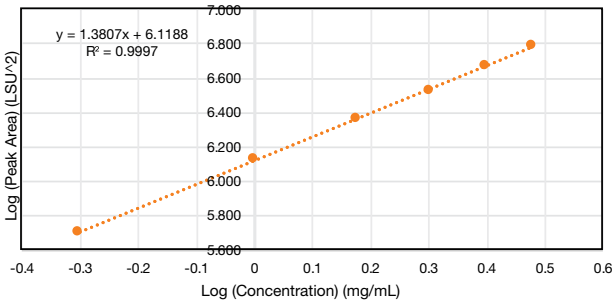
TABLE 1.

Calibrators N=5			
Standard Concentration (mg/mL)	Mean Calculated Concentration (mg/mL)	% RSD	Accuracy (%)
0	—	—	—
0.5	0.500	9.62	100
1	1.01	7.87	101
1.5	1.50	7.82	99.9
2	1.96	6.74	98.0
2.5	2.50	8.63	100
3	3.03	3.21	101

TABLE 2.

Grain Feed N=5				
Concentration Added (mg/mL)	Mean Extract Concentration (mg/mL)	Mean Content in Feed (%)	% RSD	Recovery (%)
0	0.340	1.70	8.18	—
0.5	0.869	4.35	3.13	103
1	1.39	6.95	2.34	104
1.5	1.91	9.54	2.87	104
2	2.40	12.0	2.84	102
2.5	2.91	14.6	1.59	103
3	3.36	16.8	2.46	101

Figure 6



Glucose

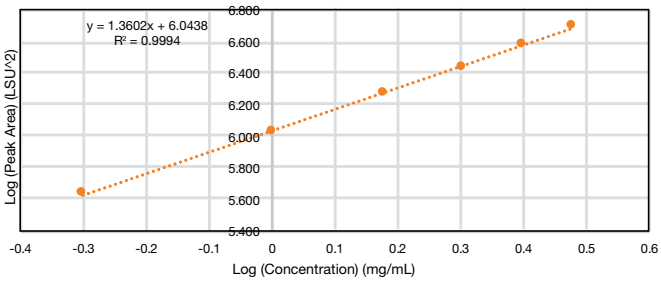
TABLE 3.

Calibrators N=5			
Standard Concentration (mg/mL)	Mean Calculated Concentration (mg/mL)	% RSD	Accuracy (%)
0	—	—	—
0.5	0.507	13.6	101
1	0.992	9.88	99.2
1.5	1.48	8.24	98.9
2	1.96	8.62	97.9
2.5	2.51	9.58	101
3	3.07	4.76	102

TABLE 4.

Grain Feed N=5				
Concentration Added (mg/mL)	Mean Extract Concentration (mg/mL)	Mean Content in Feed (%)	% RSD	Recovery (%)
0	0.257	1.28	6.58	—
0.5	0.777	3.89	4.03	103
1	1.31	6.53	3.67	104
1.5	1.82	9.11	3.21	104
2	2.35	11.8	3.33	104
2.5	2.88	14.4	1.74	104
3	3.34	16.7	3.32	103

Figure 7



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Galactose

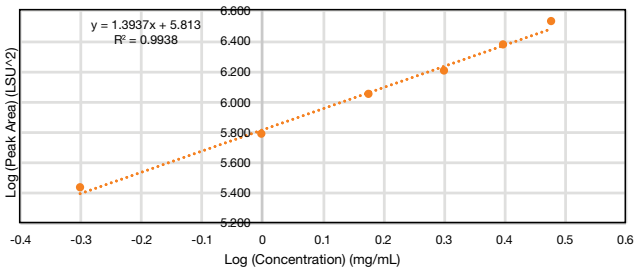
TABLE 5.

Calibrators N=5			
Standard Concentration (mg/mL)	Mean Calculated Concentration (mg/mL)	% RSD	Accuracy (%)
0	—	—	—
0.5	0.527	24.0	105
1	0.947	12.5	94.7
1.5	1.47	14.9	98.1
2	1.89	11.1	94.7
2.5	2.52	16.1	101
3	3.21	10.4	107

TABLE 6.

Grain Feed N=5				
Concentration Added (mg/mL)	Mean Extract Concentration (mg/mL)	Mean Content in Feed (%)	% RSD	Recovery (%)
0	0	0	—	—
0.5	0.637	3.19	18.6	119
1	1.18	5.92	9.00	122
1.5	1.70	8.50	14.5	114
2	2.26	11.3	7.81	116
2.5	2.80	14.0	5.61	117
3	3.23	16.2	8.86	114

FIgure 8



Sucrose

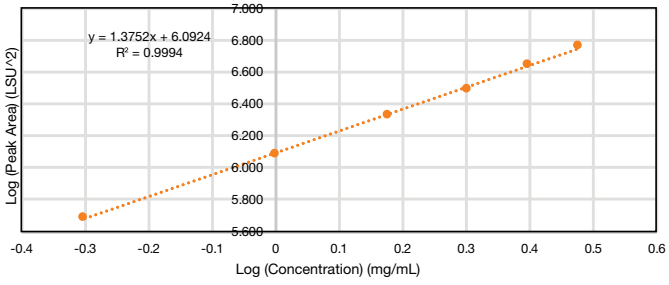
TABLE 7.

Calibrators N=5			
Standard Concentration (mg/mL)	Mean Calculated Concentration (mg/mL)	% RSD	Accuracy (%)
0	—	—	—
0.5	0.507	13.3	101
1	0.990	10.0	99.0
1.5	1.49	9.58	99.1
2	1.96	8.87	97.9
2.5	2.51	10.6	100
3	3.06	5.65	102

TABLE 8.

Grain Feed N=5				
Concentration Added (mg/mL)	Mean Extract Concentration (mg/mL)	Mean Content in Feed (%)	% RSD	Recovery (%)
0	1.061	5.30	9.41	—
0.5	1.555	7.77	5.14	99.6
1	2.042	10.2	5.40	99.1
1.5	2.555	12.8	4.16	99.8
2	3.050	15.2	4.51	99.7
2.5	3.585	17.9	3.45	101
3	4.016	20.1	2.59	98.9

Figure 9



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Maltose

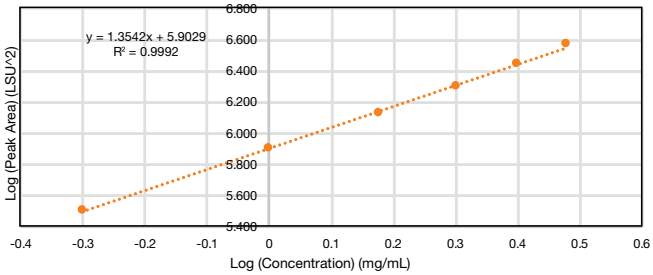
TABLE 9.

Calibrators N=5			
Standard Concentration (mg/mL)	Mean Calculated Concentration (mg/mL)	% RSD	Accuracy (%)
0	—	—	—
0.5	0.509	12.8	102
1	0.990	12.6	99.0
1.5	1.470	7.93	98.0
2	1.965	9.66	98.2
2.5	2.509	12.0	100
3	3.082	6.03	103

TABLE 10.

Grain Feed N=5				
Concentration Added (mg/mL)	Mean Extract Concentration (mg/mL)	Mean Content in Feed (%)	% RSD	Recovery (%)
0	0	0	—	—
0.5	0.554	2.77	3.68	111
1	1.057	5.29	2.22	106
1.5	1.590	7.95	3.18	106
2	2.108	10.5	3.06	105
2.5	2.663	13.3	2.13	107
3	3.148	15.7	1.58	105

Figure 10



Lactose

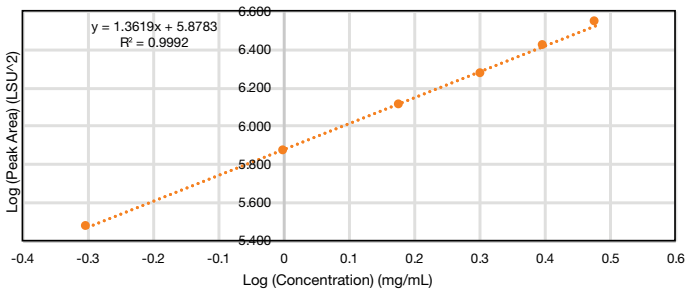
TABLE 11.

Calibrators N=5			
Standard Concentration (mg/mL)	Mean Calculated Concentration (mg/mL)	% RSD	Accuracy (%)
0	—	—	—
0.5	0.509	12.8	102
1	0.990	12.6	99.0
1.5	1.470	7.93	98.0
2	1.965	9.66	98.2
2.5	2.509	12.0	100
3	3.082	6.03	103

TABLE 12.

Grain Feed N=5				
Concentration Added (mg/mL)	Mean Extract Concentration (mg/mL)	Mean Content in Feed (%)	% RSD	Recovery (%)
0	0	0	—	—
0.5	0.535	2.67	3.54	107
1	1.054	5.27	2.15	105
1.5	1.581	7.90	2.80	105
2	2.094	10.5	2.91	105
2.5	2.651	13.3	2.58	106
3	3.125	15.6	1.87	104

Figure 11



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Results and Discussions

All samples were prepared in N=5 replicates. Calibrators were prepared in diluent composed of 50:50 ethanol/water. Fortified grain feed samples were prepared at all calibrator levels to evaluate recovery across the range. Unfortified alfalfa feed extracts tested reproducibility of the extraction and HPLC methods for quantifying inherent sugar content.

The six sugars were separated in 10 minutes using an isocratic HPLC method. Retention times were very stable with RSDs ranging from 0.06 to 1.85 %. No unresolved matrix interference peaks were observed in the extracted samples (**Figures 3** & **5**).

RSDs ranged from 3.21-13.6 % for calibrators, with the exception of galactose (**Tables 1, 3, 7, 9, 11**). Interestingly, RSDs were tighter for in-matrix samples than calibrators in diluent, with spiked samples falling between 1.59-5.40 %, with the exception of galactose (**Tables 2, 4, 8, 10, 12**). This suggests matrix-matched standards may perform better for this application.

For the calibration curves, log-log was used to account for the non-linear response of ELSD, a well-documented approach for this detector type. Linearity was excellent from 0.5-3.0 mg/mL with R^2>0.9992, with the exception of galactose (**Figures 6-11**).

Recoveries in the grain feed ranged from 98.9-111% across the concentration range, with the exception of galactose (**Tables 2, 4, 8, 10, 12**).

For galactose, calibrator RSDs ranged from 10.4-24.0% and R^2 was 0.9938 (**Table 5**). Using peak heights instead of peak areas decreased % RSDs to 2.9-10.6 % and increased the R^2 to 0.9992. These results suggest that the data processing method should be further optimized for more consistent integration of the galactose peak from the glucose peak. Additionally, using peak heights for the grain samples, galactose displayed decreasing over-recovery vs. concentration, possibly indicating the inherent glucose in the grain feed was contributing to the galactose peak but not being accounted for through standard addition calculations.

Conclusion

Demonstrated is a robust and reproducible method for the analysis of fructose, glucose, sucrose, maltose, and lactose in animal feed using HPLC-ELSD. The method employs simple sample and mobile phase preparations, and the Luna Omega SUGAR column separates six sugars in under ten minutes. Future work will include improving precision of galactose by optimizing the integration parameters.

Table 13.

Alfalfa Feed N=5			
Analyte	Mean Exteact Concentration (mg/mL)	Mean Content in Feed (%)	% RSD
Fructose	2.404	12.0	2.6
Glucose	2.171	10.9	2.1
Galactose	0	0	—
Sucrose	0.119	0.6	1.9
Maltose	0	0	—
Lactose	0	0	—

Calculations

$$\text{Total \% recovery} = 100(C_f)/(C_u + C_A)$$

Where

C_f = concentration of fortified samples
C_u = concentration of unfortified samples
C_A = concentration of analyte added to the test sample

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Ordering Information
Luna® Omega SUGAR

3 µm Minibore Columns (mm)				SecurityGuard™ Cartridges (mm)
Phases	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
				/10pk
Sugar	00B-4775-AN	00D-4775-AN	00F-4775-AN	AJ0-4496
				for ID: 2.0-3.0 mm

3 µm MidBore™ Columns (mm)		
Phases	150 x 3.0	4 x 2.0*
		/10pk
Sugar	00F-4775-Y0	AJ0-4496
		for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)				SecurityGuard Cartridges (mm)
Phases	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
				/10pk
Sugar	00D-4775-E0	00F-4775-E0	00G-4775-E0	AJ0-4495
				for ID: 3.2-8.0 mm

guarantee

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