PICKERING LABORATORIES

Analysis of Sugars in Feeds

By HPLC with Post-column Derivatization and Fluorescence Detection

The types and amounts of sugar in animal feeds are as important as the amount of protein, minerals and fats in the determination of nutritive value. We developed a simple and sensitive HPLC method for analyzing six sugars in animal feeds - Sucrose, Fructose, Glucose, Galactose, Maltose and Lactose. Post-column derivatization reagents convert reducing and non-reducing sugars into fluorescent derivatives, which greatly improves the sensitivity and selectivity of the detection.

The blends of feed examined varied from grains/vegetable products (live stock feeds) to meat/vegetable products (pet food).

Method

Sample Preparation

Mix 2.5 g of feed sample with 50 mL of water. Heat using a water bath while constantly mixing for 1 hour at 65 °C. Centrifuge and filter through 0.45 um filter.

Analytical Conditions

Column: Carbohydrate column, 4.6x150 mm

Temperature: 30 °C

Flow Rate: 1 mL/min

Mobile Phase: Acetonitrile/Water

Injection Volume: 10 uL – 50 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.4 mL

Temperature: 130 °C

Reagent 1: Guanidine hydrochloride 60 mM in 200 mM Boric acid adjusted to pH 11.5 with KOH

Reagent 2: 1.5 mM periodic acid adjusted to pH 11.5 with KOH

Flow Rate: 0.15 mL/min each reagent

Detection: FLD; λ_{ex} : 325 nm, λ_{em} : 465 nm

HPLC Gradient		
Time	Water %	ACN %
0.0	20	80
20.0	20	80
20.1	50	50
30.0	50	50
30.1	20	80

Calibration

A quadratic calibration curve with correlation > 0.999 is observed for monosaccharides such as Fructose, Glucose and Galactose. A linear calibration curve with correlation > 0.999 is observed for disaccharides such as Maltose, Lactose and Sucrose. Examples of calibration curves presented in Fig. 1 and Fig. 2.

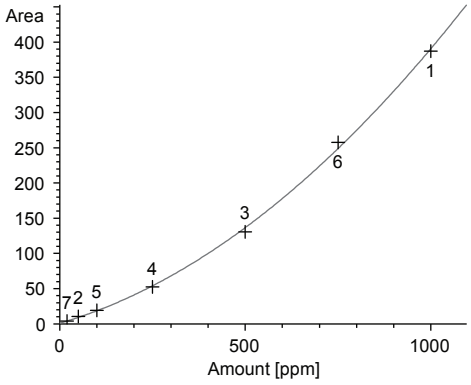

Fig 1. *Calibration curve for Frucrose.*

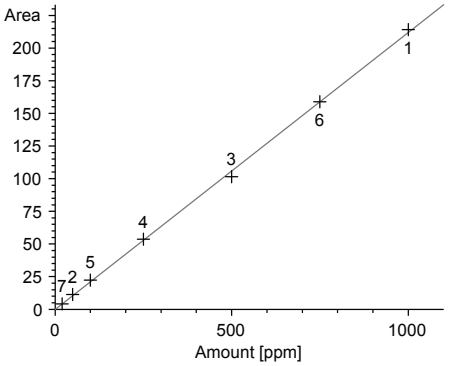

Fig 2. *Calibration curve for Maltose.*

	Fructose	Glucose	Galactose	Sucrose	Maltose	Lactose
Feed Matrix 1						
Content in feed, %	0.54	0.52	0.09	4.02	1.12	ND*
Spike Concentration, %	0.60	0.58	0.60	2.02	0.58	0.59
Recoveries, n=3, %	105	107	110	91	103	114
Spike Concentration, %	1.21	1.20	1.19	4.05	1.22	1.21
Recoveries, n=3, %	108	106	110	85	85	103
Feed Matrix 2						
Content in feed, %	0.23	0.46	ND*	3.21	0.59	ND*
Spike Concentration, %	0.42	0.57	0.56	2.50	0.57	0.58
Recoveries, n=3, %	98	101	104	106	106	107
Spike Concentration, %	0.81	1.14	1.12	4.80	1.11	1.15
Recoveries, n=3, %	103	101	101	102	102	106
Feed Matrix 3						
Content in feed, %	0.14	0.11	ND*	0.51	0.02	ND*
Spike Concentration, %	0.38	0.56	0.57	2.40	0.55	0.56
Recoveries, n=3, %	95	101	116	102	95	101
Feed Matrix 4						
Content in feed, %	0.17	0.13	ND*	1.35	0.21	ND*
Spike Concentration, %	0.41	0.57	0.58	2.43	0.56	0.56
Recoveries, n=3, %	92	97	116	101	95	102

*Not Detected

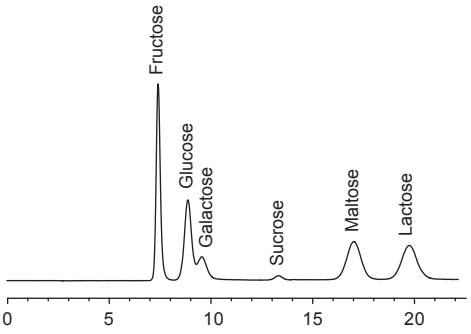

Fig 3. *Chromatogram of standard solution of sugars. Fructose 500 ppm, Glucose 500 ppm, Galactose 500 ppm, Sucrose 3000 ppm, Maltose 500 ppm, Lactose 500 ppm.*

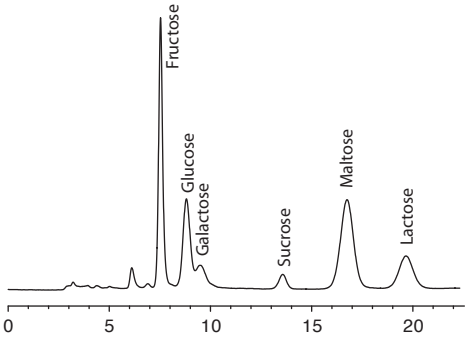

Fig 5. *Chromatogram of Feed Matrix 1 spiked with sugars. Total levels for sugars: Fructose 1.14%, Glucose 1.52%, Galactose 0.69%, Sucrose 6.02%, Maltose 1.72%, Lactose 0.6%.*

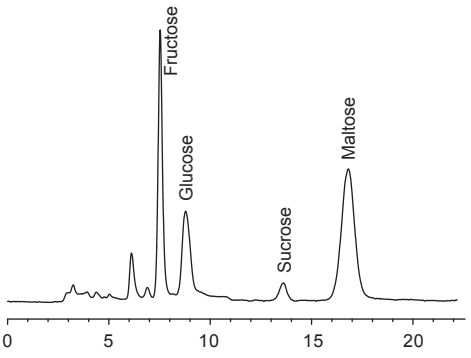

Fig 4. *Chromatogram of Feed Matrix 1. Levels of sugars present in the sample: Fructose 0.54%, Glucose 0.52%, Galactose 0.09%, Sucrose 4.02%, Maltose 1.12%.*