**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 a 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:** 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; \( \lambda_{ex} : 325 \text{ nm} \), \( \lambda_{em} : 465 \text{ nm} \)

**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.

**HPLC Gradient**

<table>
<thead>
<tr>
<th>TIME (Min)</th>
<th>WATER, %</th>
<th>ACN, %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>20</td>
<td>80</td>
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<tr>
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<td>50</td>
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<tr>
<td>30.1</td>
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<td>80</td>
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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. 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%.

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%.