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# Raffinose Family of Oligosaccharides Separated on a Hamilton HC-75

## Legumes, Blessing and Curse?

The Raffinose Family of Oligosaccharides (RFO) are low molecular weight non-reducing sugars. The sugars are connected through α-1, 6-galactosyl extensions of sucrose starting with Raffinose, Stachyose, and Verbascose. These highly water soluble sugars are universally distributed throughout the plant kingdom in roots, seeds, stems, tubers, and some leaves. RFOs are most commonly found in dehydrated legumes, like lentils, soybeans, and chickpeas, and are thought to exist mainly to protect the seed from degradation while awaiting optimal germination conditions. RFO consumption is not generally considered nutritional due to the indigestibility of α-galactosidic linkages by monogastric animals which include humans. When large amounts of under hydrated legumes are consumed by monogastric animals, multiple breakdown pathways are initiated. The gut breaks the molecules down into gases, primarily hydrogen, carbon dioxide, small amounts of methane, and short chain fatty acids! The large production of gastric gasses leads to an increase in osmotic pressure in the gut and promotes diarrhea, cramps, bloating, and overall discomfort.

As such, RFO's have generally been considered to have low nutritional value. However, studies have shown that if consumed in small quantities, < 3 g/day, positive effects occur.[2] Of these, lowered blood pressure, an increase in the production of butyrate and propionate from the gut which promote healthy brain activity, and has been shown to reduce the instance of colorectal cancer.[3] Bifidobacterium and lactobacilli concentration are shown to increase (indicated as beneficial microbiota produced from consumption of RFO's) and aid the production of short chain fatty acids, reduce gut pH, and increase the production of acetic and lactic acid which reduces pathogenic bacteria in the gut. Interestingly, various food manufacturers are now taking advantage of the beneficial aspects of bifidobacteria due to its role in colonic health.[4]

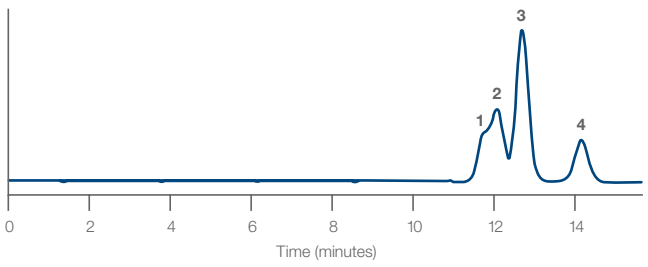
Analysis of these compounds are not easily accomplished using the more chromatographically prevalent methods (reversed-phase or ion exchange). Owing to the polar nature of this family of oligosaccharides, the Hamilton HC-75 ($Ca^{2+}$) gel matrix column was chosen. The HC-75 series uses a ligand exchange mechanism in order to interact more or less strongly with the analytes of interest. As the oligosaccharide structure increases, less optimal interaction with the exchange metal occurs resulting in a retention decrease, ending with semi overlapping peaks corresponding to the longer oligosaccharides of n = 4 and 5. In this series of saccharides, the calcium form provided the best separation. Better resolution was achieved with lower flow rates but at the cost of peak shape and time. An added advantage of the HC-75 matrix is that the mobile phase consists only of water. In this application we utilized refractive index as the detector, but conductivity or electrochemical detection can be used with good results.

### Column Information

| | |
|---|---|
| **Packing Material** | HC-75 ($Ca^{2+}$), 9 µm |
| **P/N** | 79436 |

### Chromatographic Conditions

| | |
|---|---|
| **Gradient** | Isocratic |
| **Temperature** | 80 °C |
| **Injection Volume** | 10 µL |
| **Detection** | Refractive Index |
| **Dimensions** | 305 x 7.8 mm |
| **Eluent A** | DI Water |
| **Flow Rate** | 0.6 mL/min |




**Compounds:** **1:** Verbascose **2:** Stachyose **3:** Raffinose **4:** Melibiose


1) *Cristofaro E., Mottu F., Wuhrmann J.J., Nestle Res. News, 1973, 102–104.*

2) *Mart´inez-Villaluenga C., Frias J., Vidal-Valverde C., Crit. Rev. in Food Sci. and Nut., 2008, 48:301–316.*

3) *Bruno-Barcena J.M., Azcarate-Peril M.A. J Funct Foods, 2015, 12:92–108. Mart´inez-Villaluenga C., G´omez R., Frias J., Vidal-Valverde C., J. Food Prot., 2005, 68:1246–1252.*



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