Application Note AN-P-087

# Quality labels for novel foods

## Improvement on AOAC 2001.02: GOS analysis with IC-PAD

In the past several years, interest has increased in food additives and dietary supplements including prebiotics like β-galactooligosaccharides (known as GOSs). The GOSs are chains of galactose units with an optional glucose end [1,2]. They show bifidogenic effects, i.e. they support growth and well-being of non-pathogenic gut bacteria [1]. Initially discovered as major constituents of colostrum (present up to 12 g/L), GOSs are added as a prebiotic supplement to infant formulas to achieve similar beneficial effects.

Increasing consumer awareness regarding healthy eating habits has led to the ongoing growth of global prebiotic and GOS markets. Similarly, increased demand regarding food quality has led to stricter, more comprehensive rules for food labeling and safety (e.g., EU 2015/2283). The determination of total GOS contents in food, supplements, or raw products is thus essential to fulfill such requirements.

This Application Note presents an update to the standard AOAC method for total GOS determination in foodstuffs. With the same principle (enzymatic hydrolysis of complex GOS molecules followed by chromatographic analysis of simple carbohydrates), analytical method efficiency was improved in favor of laboratory time and running costs.

## SAMPLE AND SAMPLE PREPARATION

Different commercially available samples, i.e. GOS powder (Carbosyth Ltd.), Vivinal® GOS powder (FrieslandCampina), and the supplement Bimuno Daily (Clasado Biosciences) [3], were extracted for 30 minutes at 80 °C in a phosphate buffer solution as described in AOAC 2001.02. The extract was divided into two aliquots for differential analysis of glucose, galactose, and lactose, both before (Assay 1) and after (Assay 2) enzymatic hydrolysis with the enzyme β-galactosidase from *Aspergillus oryzae* (Figure 1). Samples were centrifuged and diluted in ultrapure water (UPW) before analysis.

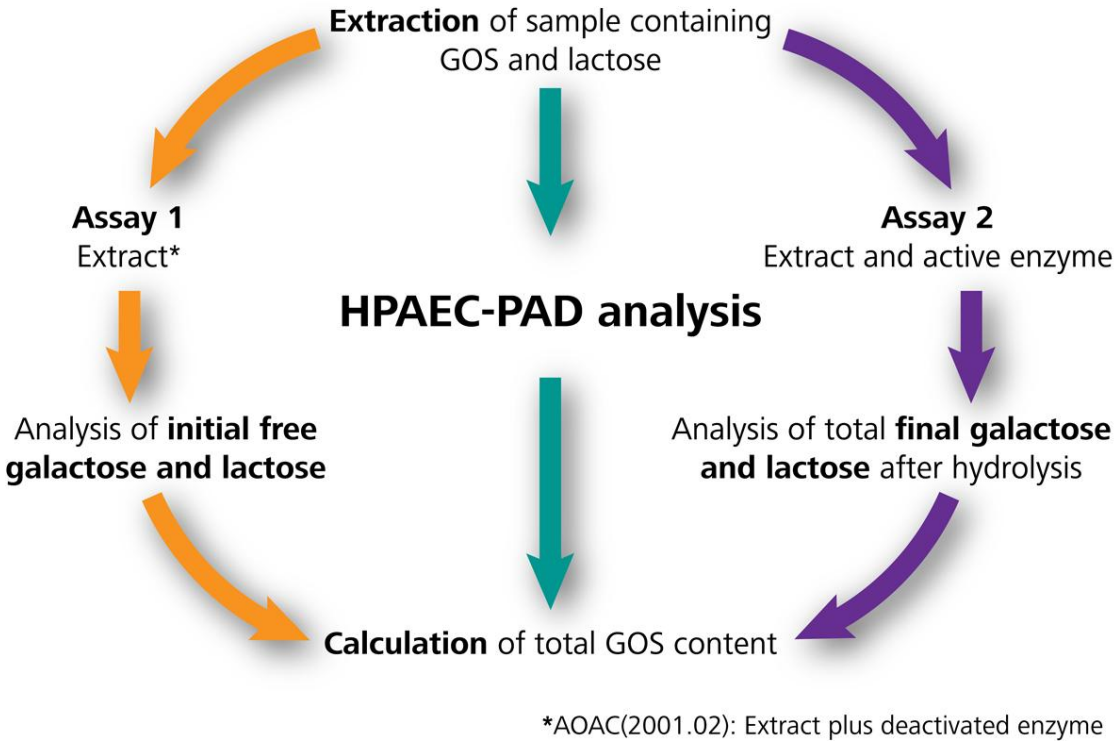


**Figure 1.** Schematic for the determination of total GOS contents using ion chromatography coupled to pulsed amperometric detection (IC-flexiPAD). Chromatography for anions in AOAC is referred as HPAEC (high performance anion exchange chromatography) but is simplified here to the generic term of IC. The improved method uses the extract for measuring of the initial glucose, galactose, and lactose concentrations (Assay 1). This was shown as equivalent to the AOAC step with the deactivated enzyme [3], but reduces chemical expenses and additional manual work. The total GOS content is calculated from the analyte concentrations in Assay 1 and Assay 2 (extract with the active enzyme). Graphic adapted from [2].

## EXPERIMENTAL

Separation of galactose, glucose, and lactose was performed on a Metrosep Carb 2 - 250/4.0 separation column using a hydroxide eluent (Figure 2) within a recording time of 18 minutes. For column clean-up, a post-recording acetate High Pressure Gradient (HPG) was run within a total sample runtime of approximately 30 minutes. Signal detection occurred with an amperometric detector (945 Professional Detector Vario - Amperometry) equipped with a Thin-Layer cell (Au working and Pd reference electrode). The use of the Thin-Layer cell in combination with a special flexiPAD (pulsed amperometric detection) waveform exhibited the best performance for the GOS analysis. Compared to the standard PAD conditions this setup provided an increased response and signal-to-noise ratio.

After manual sample preparation, Inline Dialysis with the Low Volume dialysis cell was used to purify samples before injection into the IC. As a fully automated step, proteins and larger molecules are removed from the sample matrix, protecting the column and increasing the column lifetime.

Total GOS contents were calculated after automatic data evaluation (MagIC Net 3.3 software) according to AOAC. In short, the difference of galactose and glucose between Assay 1 and Assay 2 was determined (Figure 2), corrected by initial lactose and finally adjusted to a sample weight of 100 g.

Over the course to adapt and advance the standard AOAC method, different variables were tested and finally validated according to common standards. All details are given in the open access article *Ziegler et al.* [3].

| Metrosep Carb 2 - 250/4.0 | |
|---|---|
| Eluent | 250 mmol/L NaOH, with acetate HPG rinsing step |
| Recording time | 18 min (post gradient 12 min) |
| Flow | 0.8 mL/min |
| Temp | 35 °C |
| Injection | 20 µL |
| Detection | flexiPAD |
| MISP | Dialysis |

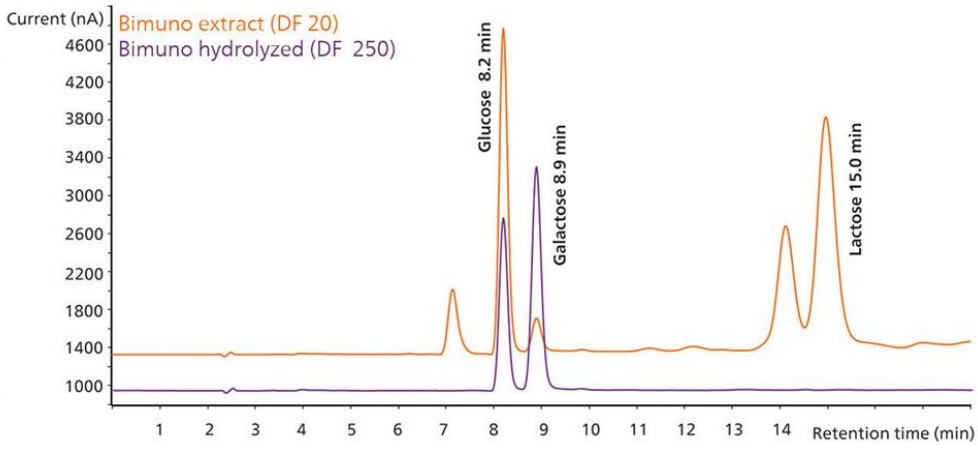


**Figure 2** Overlay (with offset) of the extract from the sample Bimuno (Assay 1, dilution factor (DF) 20 in UPW, orange) with the extract treated with β-galactosidase (Assay 2, DF 250 in UPW, purple). Due to hydrolysis of GOSs, i.e. breakdown of the galactose-galactose and galactose-glucose linkages, the concentrations of galactose and glucose in Assay 2 significantly exceed those in Assay 1. A higher DF guarantees the proper quantification within the given calibration. Chromatographic conditions are summarized on the left. As a Metrohm Inline Sample Preparation step, Inline Dialysis was used for additional sample cleanup, improving system performance and column lifetime.

## RESULTS

Total GOS contents of the different samples ranged from 28–83 g/100 g with a variability of up to 5% for measurements of individual replicates over several days. A higher variability of 6–10% was shown for infant formulas (data not shown). The higher lactose contents in such matrices results in increased uncertainties for the total GOS determination [3].

Overall, the satisfying variability, target and spike recoveries (Table 1), together with the interference tests [2], proved the method as valuable and robust. With limits of detection (LODs) (DIN 32645) of 0.1 mg/L (galactose) and 0.2 mg/L (glucose, lactose) in solution, even low total GOS contents can be determined with high precision.

**Table 1.** Total GOS contents determined with the modified AOAC 2001.02 method for the commercially available samples GOS powder, Bimuno daily supplement, and Vivinal® powder. These samples were individually prepared and analyzed in duplicate over several days (n). The calculated RSD is a measure of the variability of the total GOS contents for the different samples. Recoveries were calculated for target reference values and from spikes with the GOS powder (reference material) showing the method precision and robustness.

| Sample | Total GOS (n) (g/100 g) | Variability over n days (RSD in %) | Total GOS target (g/100 g) | Target recovery (%) | AVG Spike 1 (g/100 g) (Recovery %) | AVG Spike 2 (g/100 g) (Recovery %) |
|---|---|---|---|---|---|---|
| GOS Powder | 82.6 ± 4.1 (n = 7) | 5.0 | >70 | n.a. | n.a. | n.a. |
| Bimuno | 75.7 ± 3.0 (n = 7) | 3.9 | 79.7 | 95 | 36.8 ± 1.4 (98%) | 88.4 ± 12.7 (96%) |
| Vivinal Powder | 27.8 ± 0.5 (n = 4) | 1.8 | 28.5 | 98 | 37.8 ± 0.1 (91%) | 48.6 ± 0.1 (91%) |

## CONCLUSION

As a multicomponent method, ion chromatography with amperometric detection is a very selective, sensitive, and robust analysis method for carbohydrates without any additional derivatization steps. In combination with enzymatic treatment, even more complex carbohydrates can be quantified. The advanced and validated IC-flexiPAD method for total GOS analysis benefits analysts with enhanced efficiency. Through major sample preparation improvements, the overall procedure is not only faster by reducing additional lab work, but also reagents and consumables can be saved, lowering the total running costs. This makes it a valuable alternative to AOAC Official Method 2001.02.

Additional automation steps (e.g., Metrohm Inline Dilution and automatic calibrations) can further improve the method efficiency.

## REFERENCES


1. Sangwan et al. (2011), J. Food Sci. 76(4)
2. Boehm & Stahl (2007), J. Nutr. 137(3 Suppl 2)
3. Ziegler et al. (2001), The Column – Europe/Asia 17(02)


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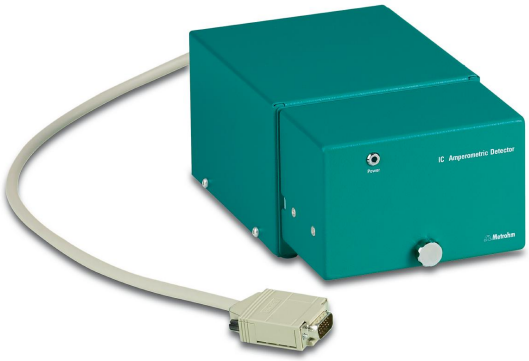
## CONFIGURATION

### 940 Professional IC Vario ONE/HPG

The 940 Professional IC Vario ONE/HPG is the intelligent IC instrument **without suppression** with **binary high-pressure gradient**. It can be extended with the 942 Extension Modules to up to a quaternary gradient system. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Carbohydrate analysis with pulsed amperometric detection (PAD) after gradient elution
- Gradient applications with UV/VIS detection with or without post-column derivatization

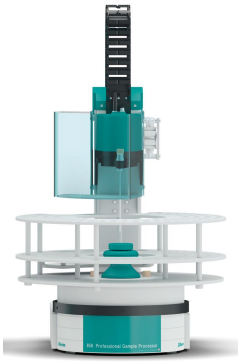



### IC Amperometric Detector

Compact and intelligent amperometric detector for intelligent IC instruments. Outstanding selectivity due to the four measuring modes: DC, PAD, flexIPAD and CV, as well as the excellent signal/noise ratio and the very fast stabilization of the measuring signal guarantee the highest in measurement precision.

### Metrosep Carb 2 - 250/4.0

The Metrosep Carb 2 - 250/4.0 IC column is particularly suitable for the determination of carbohydrates using alkaline eluents and pulsed amperometric detection. The high-capacity anion exchanger column is based on a styrene-divinylbenzene copolymer. It is stable in the range of pH = 0 - 14 and separates monosaccharides and disaccharides. It is also suitable for the analysis of sugar alcohols, anhydrous sugars, amino sugars, etc. The 250 mm version of the Metrosep Carb 2 separation column is optimized for complex separations.



### 858 Professional Sample Processor – Pump

The 858 Professional Sample Processor – Pump processes samples from 500 µL to 500 mL. The sample transfer takes place either with the installed bidirectional two-channel peristaltic pump or with an 800 Dosino.

MagIC Net 4.0 Professional: 1 license

Professional PC program for controlling all intelligent Professional IC systems, Compact IC systems and their peripherals, all detectors and various Auto samplers, 800 Dosino, 771 Compact Interface, etc. The software permits checks, data acquisition, data evaluation and data monitoring as well as report generation of ion chromatographic analyses.

Graphical user interface for routine operations, extensive database programs, method development, configuration and manual system control; very flexible user management, powerful database operations, extensive data export functions, individually configurable report generator, control and monitoring of all system components and the chromatography results.

MagIC Net Professional complies fully with FDA Regulation 21 CFR Part 11 as well as GLP.

MagIC Net is available in 16 dialog languages: German, English, Chinese, Traditional Chinese, French, Italian, Spanish, Portuguese, Bulgarian, Czech, Hungarian, Japanese, Korean, Russian, Slovakian, Polish

1 license

The installation and documents are supplied on a USB flash drive.