



Advanced Laser Polarimeter (ALP) Carbohydrates (Sugars):

Detection of Carbohydrates (Sugars)

HPLC is an important tool for identification and quantitation of carbohydrates in foods, beverages, and different types of natural samples. Carbohydrate analyses are challenging because of potential complexity in molecular structures and similarity of physico-chemical properties among a group of similar compounds.

The UV detection of carbohydrates is limited due to absence of sufficient absorption and necessity of derivitization. Low-wavelength UV (190 – 205nm) lacks sensitivity and places strict requirements on the optical properties of the mobile phase.

Traditional detection techniques are RI (refractive index) and ELSD (evaporative light scattering detector). A major disadvantage of RI is poor sensitivity and baseline instability. In complex mixtures the sample components may cover a wide range of refractive index values and may closely match that of the mobile phase, becoming invisible to the detector. Any change in eluent composition requires rebalancing of RI detectors, thus not recommended with gradients.

ELSD offers independence from chemical structure, but is not a universal technique. Limitations in sensitivity and dynamic range and lack of consistent response make carbohydrate analysis difficult.

These problems in carbohydrate detection can easily be overcome by using our ALP (Advanced Laser Polarimeter).

- **Universal Detection:** No requirement for analyte to have a chromophore, only optical activity. Any optically active compound separated on the column will be detected. Compounds with positive and negative specific rotation will elute as positive and negative peaks.
- **Very Good Sensitivity:** Sub ng limits of detection (ca. 0.5 ng for D-glucose). Increased sensitivity response with increasing absolute value of optical rotation.
- **Broad Applicability:** Can be used with wide range of HPLC conditions (solvents, temperature, flow) in normal and reverse phase, independent from solvent properties.
- **Good Reproducibility:** In most cases RSD is less than 2%.
- **Wide Dynamic Range:** ng to mg injections in analysis.
- **Easy, Simple to Use, Automated Operation:** Can be set up the same way as an in-line UV detector.
- **Compatible with any HPLC system.**

ALP is used from method development & analysis to preparative purification to process control.

Several experiments demonstrating capabilities of process control using polarimetric detection were conducted with conversion of glucose to gluconic acid. The depletion of reactant could be easily followed using ALP as a detector, when the start compound was converted into a product with different chirality.

Basically, if an optically active carbohydrate can be separated by HPLC/SFC, it will be detected by ALP.

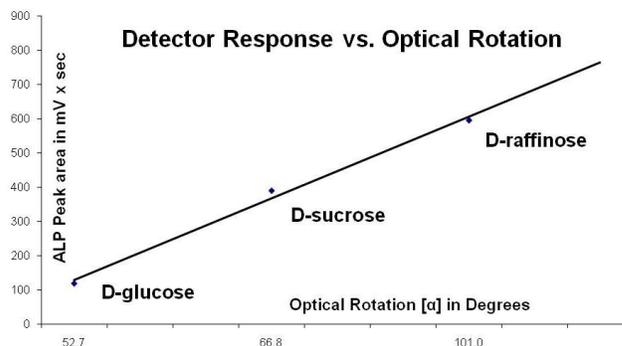


Figure 1: D-glucose, D-sucrose, D-raffinose (each sample = 0.01M in Water)

Injection Number	Peak area mV x sec	RSD %
1	111.49	0.7
2	111.91	
3	112.14	
4	110.12	
5	110.86	

Figure 2: RSD for D-glucose
 0.01M in Water
 Rexchrome Amino 150 x 4.6mm x 5μ
 Eluent = Water

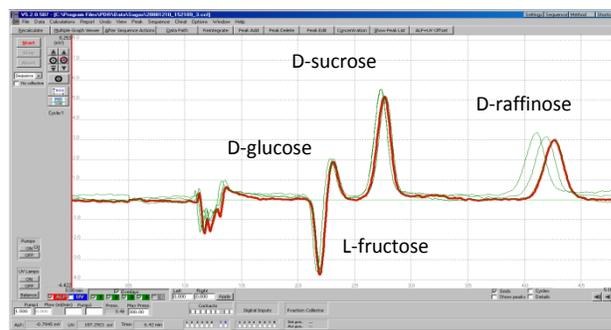


Figure 3: Reproducibility of ALP with Four sugars
 L-fructose, D-glucose, D-sucrose, D-raffinose
 1 mg/mL each in Water, injected 4 times
 Rexchrome Amino 150 x 4.6mm x 5μ
 Eluent = Acetonitrile/Water 72/28