Optimized HPLC-DAD methodology for the rapid quantification of monosaccharides in complex matrices

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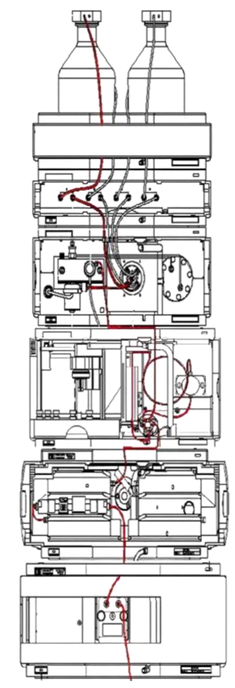
Introduction:

Reducing sugars, particularly aldoses with cyclic structures, exhibit strong reactivity toward 1-phenyl-3-methyl-5-pyrazolone (PMP), forming sugar-PMP derivatives that can be detected by ultraviolet (UV) detection at 248 nm. Commonly used methods often fail to accurately identify and quantify xylose and arabinose due to their co-elution, resulting in a single unresolved peak. In this study, seven monosaccharides (mannose, rhamnose, galacturonic acid, glucose, galactose, xylose and arabinose) were chromatographically separated using a C18 column by HPLC-DAD. **The aim of the study** is to optimize the conventional method to prevent co-elution and reduce overall analysis time.

Methods:



- Mobile phase A optimized to ammonium acetate within a range of 25 – 75 mM and pH 3 – 7.5
- Flow changed from 0.3 mL/min to 0.85 mL/min.
- Analysis time reduced from 40 to 15 min
- Column temperature lowered from 40ºC to 15ºC

Results:

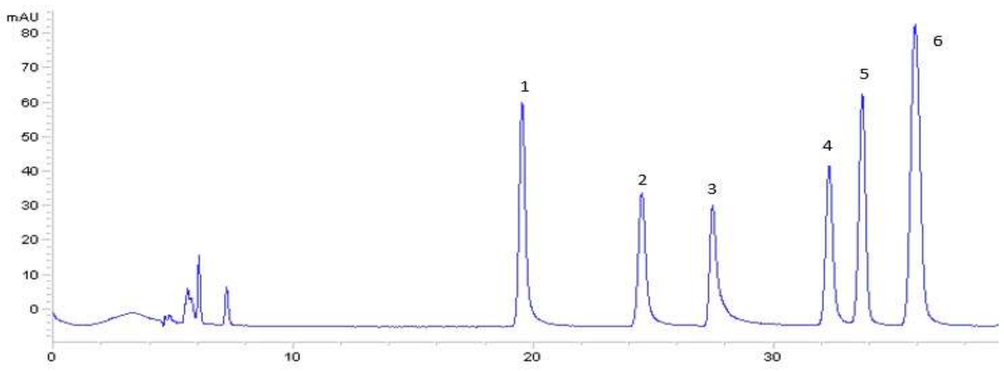


Figure 1. Chromatogram of the seven monosaccharides. Flow rate was set at 0.3 mL/min, column temperature was at 40°C, the total analysis time was 40 minutes, and concentration of mobile phase A was 50 mM at pH 5.5. Resolution of D-Mannose (1), D-Ramnose (2), D-Galacturonic acid (3), D-Glucose (4), D-Galactose (5) and co-elution of D-Xylose and L-Arabinose (6).

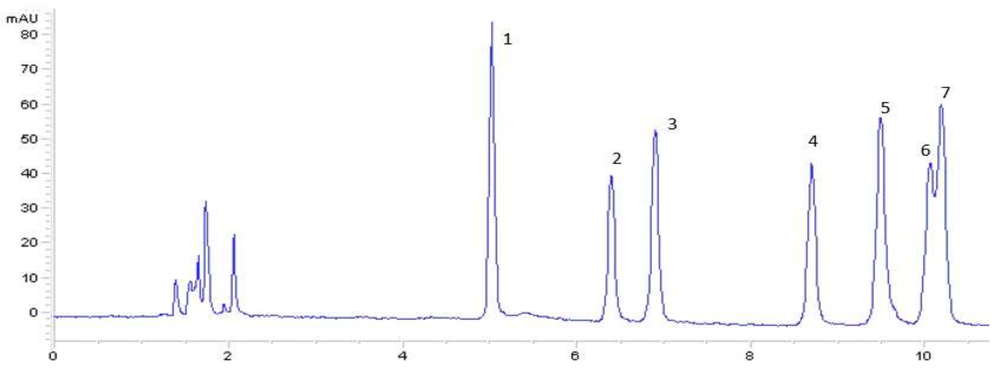


Figure 2. Chromatogram of the seven monosaccharides. Flow rate was set at 0.6 mL/min, column temperature was at 40°C, the total analysis time was 15 minutes, and concentration of mobile phase A was 50 mM at pH 7.5. Resolution of D-Mannose (1), D-Ramnose (2), D-Galacturonic acid (3), D-Glucose (4), D-Galactose (5), D-Xylose (6) and L-Arabinose (7).

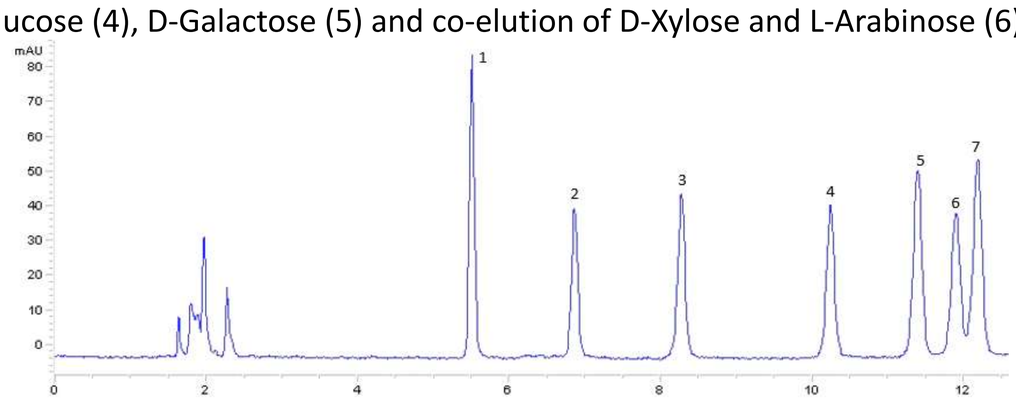


Figure 3. Chromatogram of the seven monosaccharides. Flow rate was set at 0.6 mL/min, column temperature was at 15°C, the total analysis time was 15 minutes, and concentration of mobile phase A was 50 mM at pH 7.5. Resolution of D-Mannose (1), D-Ramnose (2), D-Galacturonic acid (3), D-Glucose (4), D-Galactose (5), D-Xylose (6) and L-Arabinose (7).

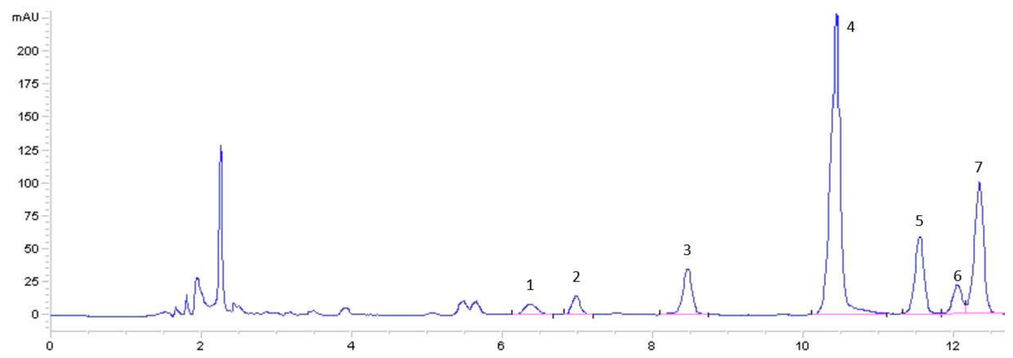


Figure 4. Chromatogram of the seven monosaccharides in a byproduct of lemon. Flow rate was set at 0.6 mL/min, column temperature was at 15°C, the total analysis time was 15 minutes, and concentration of mobile phase A was 50 mM at pH 7.5. Resolution of D-Mannose (1), D-Ramnose (2), D-Galacturonic acid (3), D-Glucose (4), D-Galactose (5), D-Xylose (6) and L-Arabinose (7).

Conclusions:

Following the optimization process, the variations of chromatographic conditions resulted in a reduction of the total analysis time to 15 minutes compared to the original protocol. Moreover, a complete resolution between the xylose and arabinose peaks was achieved, allowing a proper identification and quantification.