Avantor Korea | FOCUS: LIFE SCIENCE 2024 December

Email: customerservicekr@avantorsciences.com

Tel: 031-645-7250

Creating the Correct Environment for Analyzing Reducing Sugars with the Avantor ACE® NH_2

Avantor ACE® NH_2 활용을 통해 당류 감소 분석에 적합한 환경 조성 방법

Mark Fever, R&D Manager
Colin Pipe, Senior Research Scientist

Abstract

This white paper focuses on achieving accurate and consistent analysis of reducing sugars through High-Performance Liquid Chromatography (HPLC) using the ACE® NH_2 column. It highlights challenges such as controlling environmental factors, optimizing separation conditions, and ensuring precision in complex sugar matrices. The ACE® NH_2 column's performance is emphasized for its ability to deliver robust, reproducible results, providing practical insights and best practices for researchers in sugar analysis.

Keywords

Reducing Sugars, Chromatographic Analysis, Environmental Control, HPLC, High-Performance Liquid Chromatography

Chromatography Solutions

Knowledge note #0022

Creating the Correct Environment for Analysing Reducing Sugars with the Avantor® ACE® NH_2

INTRODUCTION

Sugars are carbohydrates composed of carbon, hydrogen and oxygen. In cyclic form, they are capable of forming isomers known as anomers at the acetal carbon (known as the anomeric carbon). Anomerisation is the conversion of one anomer to the other and readily occurs whilst in solution. Under non-ideal chromatographic conditions, the anomeric forms can be observed as two peaks. This Knowledge Note explains the anomerisation process and how to create the correct environment for analysing reducing sugars using the Avantor® ACE® Excel NH_2.

SUGAR CHEMISTRY

Reducing sugars contain an anomeric carbon that is able to convert between the α- and β-anomeric forms. A non-reducing sugar is one which has used the anomeric carbon to form the glycosidic bond and therefore cannot interconvert. All monosaccharides and some disaccharides are reducing sugars. LC analysis of sugars is often performed using an amino column.

Under certain conditions, it is possible to observe both anomeric forms of reducing sugars (e.g. peak 1 = fructose in Figure 1(a)). Chromatographically, this results in broad peaks, often showing a distinct shoulder, or split peaks. This is due to interaction of the separate anomers with the positive charge on the amino ligand ($-NH_3+$).

When washed with weak ammonia, using the protocol described in this document, the stationary phase charge is removed to generate the non-protonated amino form ($-NH_2$). The non-protonated amino phase facilitates fast anomerisation, dramatically improving the chromatography obtained (e.g. fructose, peak 1 in Figure 1(b)).

Sucrose (Peak 2) is a non-reducing sugar (i.e. no anomeric hydroxyl group) and is therefore observed as a single peak in both analyses.

The flushing protocol outlined on the following page should be used when analysing reducing sugars to ensure a single peak is obtained.

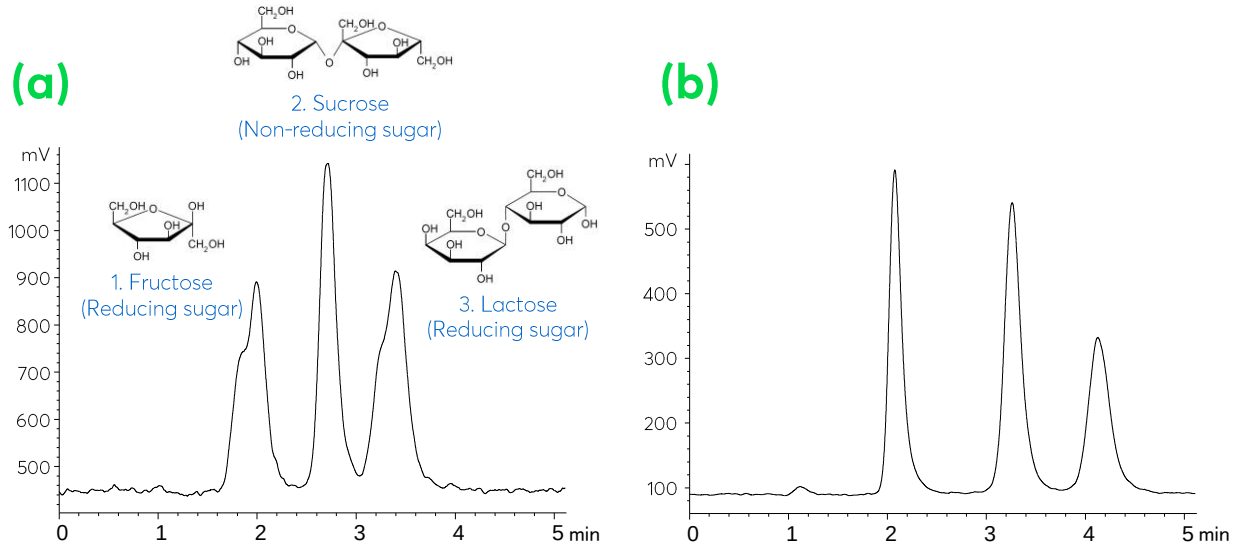

Figure 1: Comparison of the peak shape obtained for the analysis of sugars on the Avantor® ACE® Excel 5 NH_2 (50 x 2.1 mm) before (a) and after (b) flushing with 0.1% NH_3 in MeCN/H_2O (75:25 v/v).
Mobile phase: MeCN/H_2O (75:25 v/v); Flow rate: 0.21 mL/min; Temperature: 35°C; Detection: ELSD.

AVANTOR® ACE® NH_2 FLUSHING PROTOCOL FOR SUGAR ANALYSIS

Prior to first use, column **MUST** be flushed as follows:

- 20 column volumes of 7:3 v/v MeCN/H_2O
- 50 column volumes of 7:3 v/v MeCN/H_2O + 0.1% v/v ammonia solution (32% approx.)
- 20 column volumes of 7:3 v/v MeCN/H_2O

CONCLUSION

This Knowledge Note has explained the need to create the correct amino stationary phase environment for sugar analysis. Simply flushing the Avantor® ACE® Excel NH_2 prior to first use with a 0.1% w/w ammonia in aqueous:organic eluent is recommended. Failing to do so will result in poor peak shape for reducing sugars due to anomerisation.

vwr.com/ace
chromsupport@avantorsciences.com