Creating the Correct Environment for Analysing Reducing Sugars with the ACE NH$_2$

ABSTRACT
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). Anomerization 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 ACE Knowledge Note explains the anomeric process and how to simply create the correct environment for analysing reducing sugars using ACE Excel NH$_2$.

INTRODUCTION

In sugar chemistry, all monosaccharides and some disaccharides are reducing sugars.

Reducing sugars contain an anomic carbon that is able to convert between the α- and β-anomeric forms. A non-reducing sugar is one which has used the anomeric carbons to form the glycosidic bond and therefore cannot interconvert.

Under certain conditions, it is possible to observe both anomers for reducing sugars (e.g. peak 1 = fructose in Figure 1(a)). The peaks are a result of the separate anomers interacting with the positive charge on the amino ligand (-NH$_3$⁺).

When washed with weak ammonia using the protocol below, the stationary phase charge is removed giving -NH$_2$. The unprotonated amino phase facilitates fast anomerisation and only one peak is observed for the analysis (e.g. fructose, peak 1 in Figure 1(b)).

Sucrose (Peak 2) is a non-reducing sugar (i.e. no anomeric hydroxyl group) therefore only produces one peak in both analyses.

The flushing protocol suggested herein should be used when analysing reducing sugars to ensure a single peak is obtained.
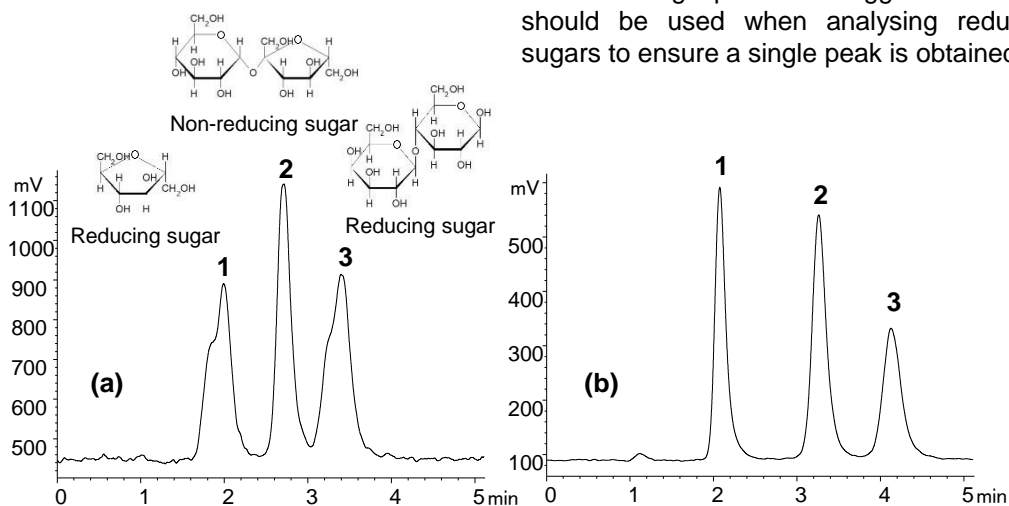

Figure 1 Comparison of sugars on the ACE Excel 5 NH$_2$ (50 x 2.1 mm) before and after flushing with 0.1% NH$_3$ in MeCN/H$_2$O (75:25 v/v), 0.21 mL/min, 35°C, RID. Peak 1: Fructose, Peak 2: Sucrose, Peak 3: Lactose

Prior to first use, column MUST be flushed as follows:
1. 20 column volumes of 7:3 v/v MeCN/H$_2$O
2. 50 column volumes of 7:3 v/v MeCN/H$_2$O + 0.1% v/v ammonia solution (32% approx.)
3. 20 column volumes of 7:3 v/v MeCN/H$_2$O

CONCLUSION
This ACE Knowledge Note has explained the need to create the correct amino stationary phase environment for sugar analysis. Simply flushing the 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 the anomerization process.