

## Application Note

# Determination of Food Sugars in Fruit Juice Using Refractive Index Detection and BEH Amide Column Chemistry

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This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

XBridge BEH Amide column chemistry provided an analysis time of less than eight minutes for the analysis of sugars in fruit juice. By employing isocratic elution, the equilibration requirement between injections was not necessary. This method enabled the accurate quantification of sugars in fruit juices using robust XBridge BEH Amide XP column chemistry. Proven Alliance HPLC System technology with accurate solvent delivery, repeatable injection performance, precise column temperature control, and simple RI detection, meets the goals of simple sample preparation, rapid analysis time, and cost effectiveness.

## Benefits

- Isocratic elution using proven Alliance HPLC technology with XBridge BEH Amide eXtended Performance [XP] column chemistry and refractive index detection results in a simple, rapid, and cost effective system for the quantification of sugars in fruit juice.

- Successful determination of sugar content in a variety of fruit juices with minimum sample preparation, rapid analysis time, and reduced costs.

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## Introduction

The healthier option of consuming pure fruit juice instead of soft drinks containing sugar has been promoted in recent years due to studies linking the latter with obesity, coronary problems, and the development of type 2 diabetes. In addition, certain juices such as pomegranate have been reported to provide additional health benefits due to their antioxidant content. These products, therefore, often command premium prices.

To ensure quality and satisfy regulatory agencies, the fruit juice industry must test these products to meet certain standards. One indicator is sugar content. Fructose, sucrose, and glucose are important analytes in fruit juice analysis. Their concentration and relative ratio vary and serve as markers for different cultivars.

HPLC with refractive index (RI) detection has long been the recognized technique for these analyses. Columns for sugar separations have often used amine-based chemistry. However, this can lead to the formation of Schiff bases that can shorten column lifetime and lead to inaccurate sugar quantification.

Using Waters proprietary amide column chemistry, Schiff bases are avoided, thereby eliminating the aforementioned problems. In this study, we show the versatility of the Waters XBridge Amide *XP* column for sugar analysis.



*Figure 1. Alliance HPLC System with 2414 Refractive Index Detector.*

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## Results and Discussion

Various fruit juices were purchased in local markets. Samples of these juices were centrifuged at a high speed to remove pulp and other particulate matter. A portion of the supernatant of each juice was diluted with a 1:50 water/acetone mixture. This was the only sample preparation required.

The analyses were performed on an Alliance HPLC System equipped with a 2414 Refractive Index (RI) Detector. RI detection is appropriate here, as the target sugar analytes have no chromophores, eliminating the feasibility of using UV or fluorescence detection. The thermally isolated optics bench provides a sensitive, stable signal able to measure changes in refractive index independent of ambient temperature changes. In this study, an XBridge BEH Amide *XP* 4.6 x 100 mm, 2.5  $\mu$ m Column was used. Isocratic elution was used with a mobile phase consisting of water and acetone modified with triethyl amine (TEA). Samples were quantified against a six-point

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calibration curve generated by running dilutions of a stock sugar mixture. Figure 2 shows the separation of five food sugars, while a profile of several fruit juices is shown in Figure 3.

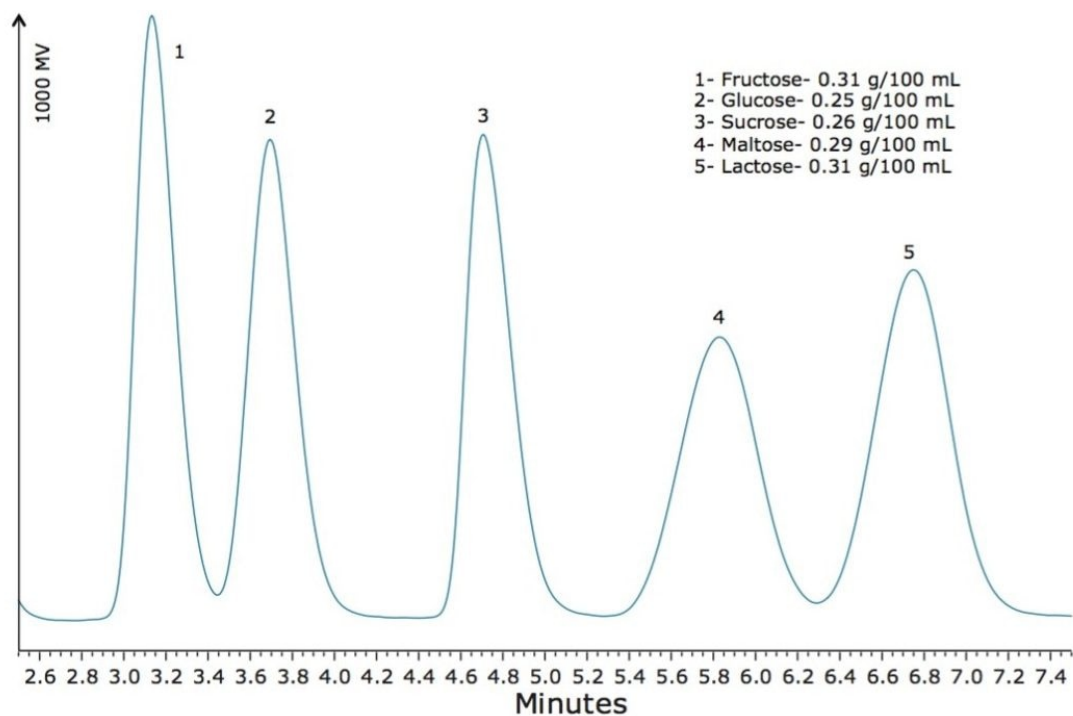


Figure 2. RI detection chromatogram showing the separation of the five food sugars.

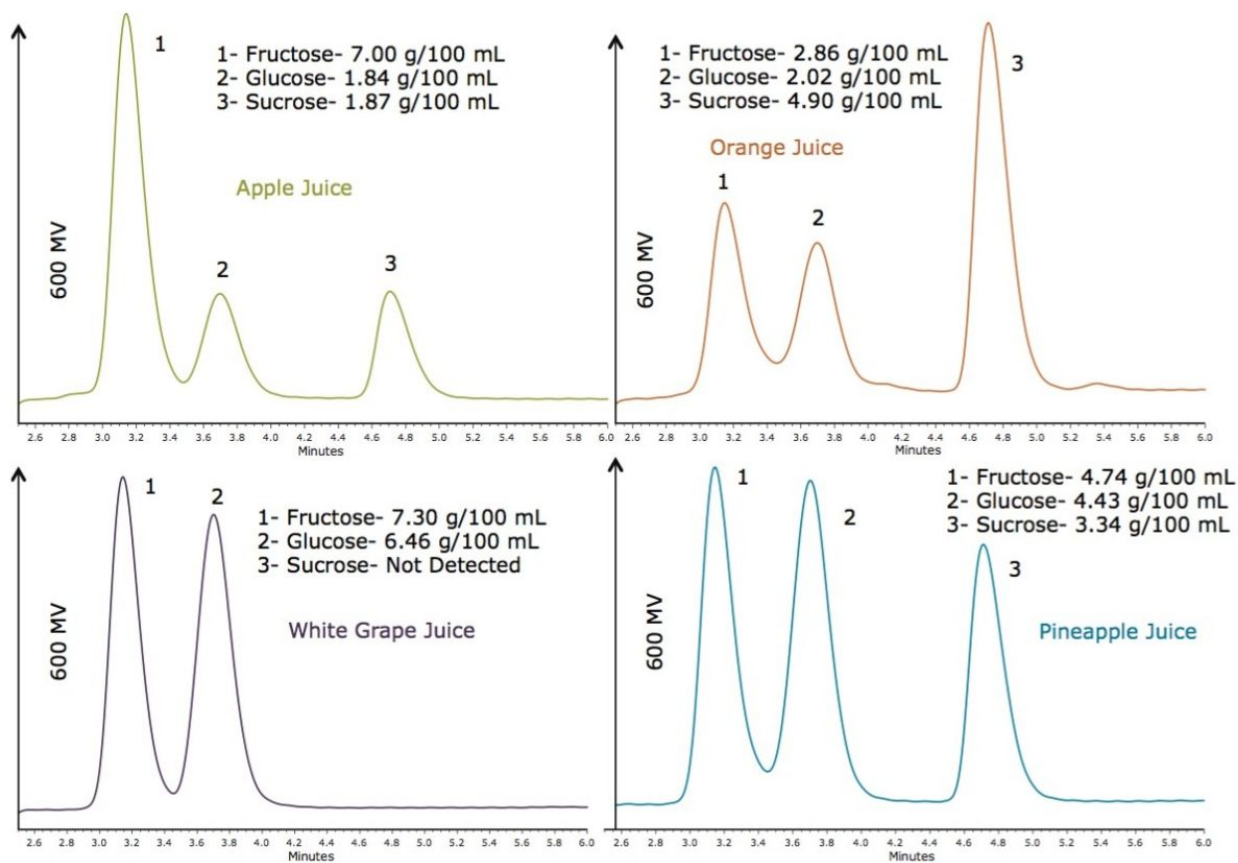


Figure 3. Sugar profiles of four fruit juices. Calculated amounts of different sugars are labeled with the 50x dilution in sample preparation taken into account.

## Conclusion

XBridge BEH Amide column chemistry provided an analysis time of less than eight minutes for the analysis of sugars in fruit juice. By employing isocratic elution, the equilibration requirement between injections was not necessary. This method enabled the accurate quantification of sugars in fruit juices using robust XBridge BEH Amide *XP* column chemistry. Proven Alliance HPLC System technology with accurate solvent delivery, repeatable injection performance, precise column temperature control, and simple RI detection, meets the goals of simple sample preparation, rapid analysis time, and cost effectiveness.

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## Featured Products

Alliance HPLC System <<https://www.waters.com/534293>>

2414 Refractive Index (RI) Detector <<https://www.waters.com/514425>>

## Available for purchase online

XBridge BEH Amide XP Column, 130Å, 2.5 µm, 4.6 mm X 100 mm <  
<https://www.waters.com/waters/partDetail.htm?partNumber=186006099>>

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