

# Simplified scale up for sugars with the AZURA® RID 2.1L extended dynamic range option



Juliane Böttcher, Kate Monks; [applications@knauer.net](mailto:applications@knauer.net)

KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; [www.knauer.net](http://www.knauer.net)

## SUMMARY

The extended dynamic range (EDR) option for the AZURA® RID 2.1L refractive index detector was investigated with a simple sample consisting of two common sugars. Calibration curves covering the range 700  $\mu$ RIU to 2300  $\mu$ RIU were generated with activated and deactivated EDR. A gain of about 65 % in dynamic range could be demonstrated over this range. Further benefits, such as simplified sample preparation, and improved fractionation possibilities are also discussed.

## INTRODUCTION

The extended dynamic range (EDR) option of the AZURA RID 2.1L enables the linear dynamic range to be broadened in +100 % (-1000  $\mu$ RIU offset) or -100 % (+1000  $\mu$ RIU offset) [1]. This feature enhances the application of this detector for semi-preparative, preparative, and scale-up purposes. For instance, when carrying out overload studies, it is necessary to know how much sample and at which concentration

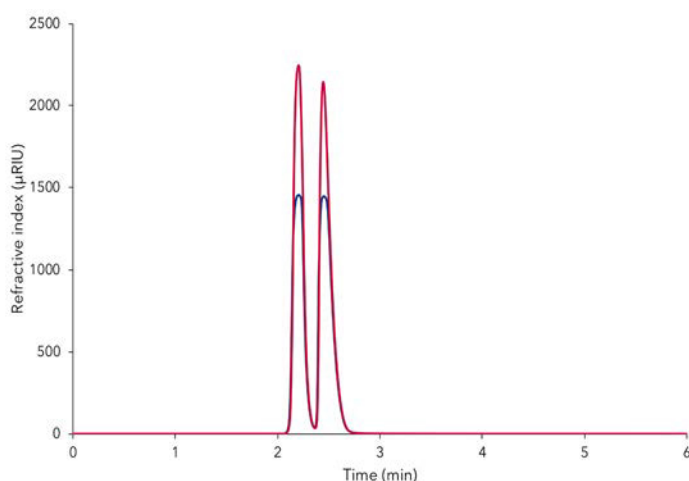
can be injected on an analytical column. Often these measurements are out of the detector's linear dynamic range. The EDR feature is very useful in this case because it enables the more exact calculation of the amount of sample that can be loaded on a column for purification without the need for additional sample preparation.

# Simplified scale up for sugars with the AZURA® RID 2.1L extended dynamic range option

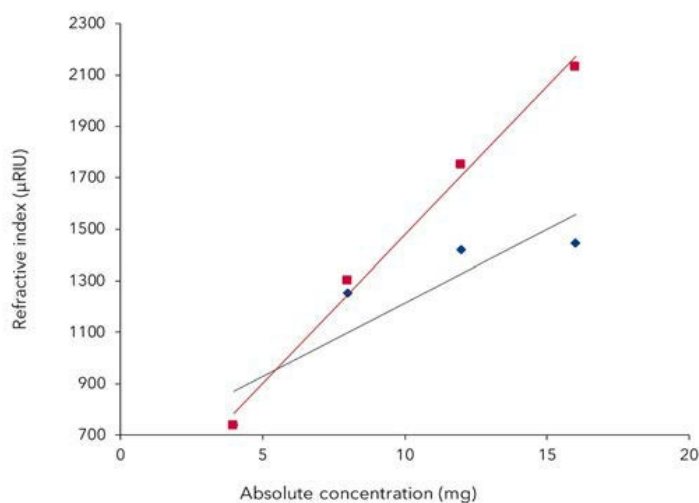
## RESULTS

To investigate the influence of the EDR option a simple method was chosen. A solution with a concentration of 40 mg/mL glucose and saccharose was injected with different volumes (10  $\mu$ L, 20  $\mu$ L, 30  $\mu$ L, 40  $\mu$ L, 50  $\mu$ L, 100  $\mu$ L, 200  $\mu$ L) and measured with and without activated EDR. **Fig 1** shows an overlay of an injection of 50  $\mu$ L with and without extension. The blue trace is without extension, the red trace is detected using the

+100% option. When using the extension, a better resolution was gained as well as a higher and sharper signal was achieved. Now it was possible to measure up to 2.5 mRIU without difficulty. The advantage of the EDR option due to the linearity of calibration is visualized in **Fig 2**. It is shown that when using the extension, better values of linearity and correlation coefficient can be achieved over a wide range.



**Fig. 1** Overlay of an injection with 50  $\mu$ L, blue = without extension, red = with extension (+100%)



**Fig. 2** Linearity of glucose calibration with (red, R=0.9924) and without (blue, R=0.8087) EDR option

## MATERIALS AND METHOD

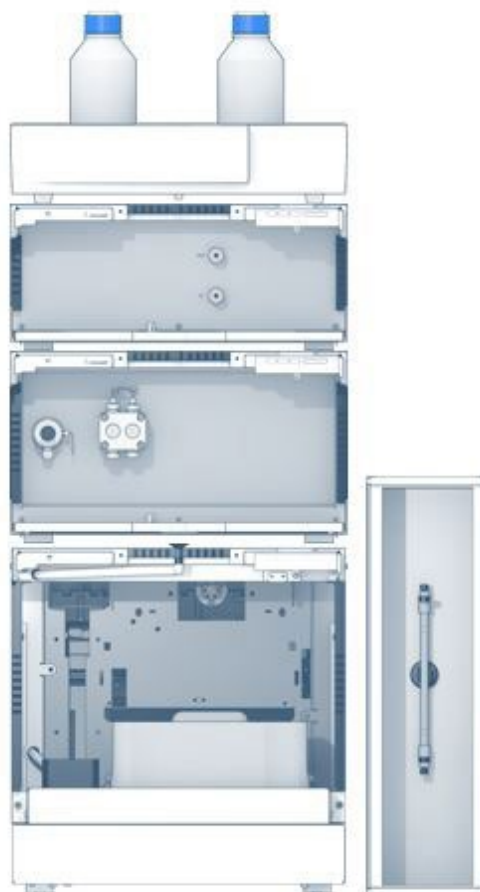
An AZURA® Analytical HPLC Plus system was used for this application. The system consisted of an isocratic AZURA P 6.1L pump, an AZURA AS 6.1L autosampler, an AZURA CT 2.1 column thermostat and an AZURA RID 2.1L refractive index detector. The used column was filled with Eurospher II 100 5 C18A silica. The isocratic method ran at a flow rate of 1.0 mL/min with a 100 % aqueous eluent for 6 minutes. The column thermostat was set to 25 °C and the data rate of the detector to 20 Hz. Different volumes (10 µL, 20 µL, 30 µL, 40 µL, 50 µL, 100 µL, 200 µL) of a solution containing 40 mg/mL of glucose and saccharose were injected.

## CONCLUSION

The EDR feature was shown to prevent the need to dilute samples, which saves time and money and diminishes additional errors during sample preparation. Furthermore, due to an improved peak shape at high sample concentrations, software fractionation algorithms can work more efficiently. Therefore this feature facilitates a more efficient purification. Here, the EDR was used in positive mode (+100%). For applications with inverted peaks, similar applicative benefits could be achieved by activating the negative mode EDR (-100%). This was not carried out in this study.

## REFERENCES

[1] <https://www.knauer.net/certificates>



## ADDITIONAL MATERIALS AND METHODS

Tab.A1 Method parameters

Eluent A	H <sub>2</sub> O <sub>dd</sub>		
Gradient	Isocratic, 100 % A		
Flow rate	1 mL/min	Run time	6 min
Column temperature	25 °C	Injection mode	Partial loop/Full loop
Injection volume	10 µL, 20 µL, 30 µL, 40 µL, 50 µL, 100 µL, 200 µL	Data rate	20 Hz
		Time constant	0.05 sec

Tab.A2 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L, isocratic, 10 mL, SS	<a href="#">APH30EA</a>
Autosampler	AZURA AS 6.1L	<a href="#">AAA00AA</a>
Detector	AZURA RID 2.1L	<a href="#">ADD31</a>
Thermostat	AZURA CT 2.1	<a href="#">A05852</a>
Eluent tray	AZURA E 2.1L	<a href="#">AZC00</a>
Column	Vertex Plus Column, 250x4 mm, Eurospher II 100-5 C18A with precolumn	<a href="#">25WE184E2J</a>
Software	ClarityChrom 6.1.0	<a href="#">A1670-9</a>

## RELATED KNAUER APPLICATIONS

**VFD0160** - Determination of sugars and natural sugar substitutes in different matrices

**VFD0161** - Determination of sugars in honey using HILIC separation and RI detection

**VFD0155** - Semi preparative xylitol purification with dedicated sugar purification system

**VFD0150** - Alternative xylitol extraction via hplc purification from fermented biomass