

## Separation of Mono and Disaccharides on ACQUITY Arc System with New Column Compartments

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Jinchuan Yang

Waters Corporation



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

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## Abstract

This application brief demonstrates successful separation of the critical pair of galactose and glucose in a Waters ACQUITY Arc System with CH-30A, CH-A, or CM-A column compartment options.

### Benefits

The ACQUITY Arc System with new column compartments performs equivalently to the original configuration for sugar analysis.

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## Introduction

Sugar analysis that uses a Waters XBridge BEH Amide *XP* Column and a Waters ACQUITY QDa Mass Detector has recently been developed on an ACQUITY Arc System with a CH-30A Column Heater.<sup>1</sup> In this method, the content of six common sugars in foods, specifically fructose, galactose, glucose, sucrose, lactose, and maltose, can be accurately and reliably determined within a 25-min injection cycle time. One of the key advantages of this new method is the chromatographic separation of a critical pair of sugar compounds, the galactose and the glucose. Resolution of 1.2 can be achieved, while in other methods these two compounds are difficult to be separated.

New column compartment options, which offer more flexibility, have recently become available to the ACQUITY Arc System to meet versatile analytical needs. The CH-A Column Heater offers reduced footprint compared to the original CH-30A Column Heater, and the CM-A Column Manager provides a wide temperature range and expanded column capacity (see Figure 1). These new column compartments have different fluidic paths, which might affect the separation of sugars, especially the critical pair. Since the separation resolution for galactose and glucose is one of the key performance criteria in sugar analysis,<sup>1</sup> it is necessary to evaluate the separation of the critical pair in those new column compartments. In this technology brief, we demonstrate that the ACQUITY Arc System with the new column compartment options, the CH-A Column Heater and the CM-A column manager, performs equivalently to the original column compartment configuration, the CH-30A Column Heater, in separating galactose and glucose.

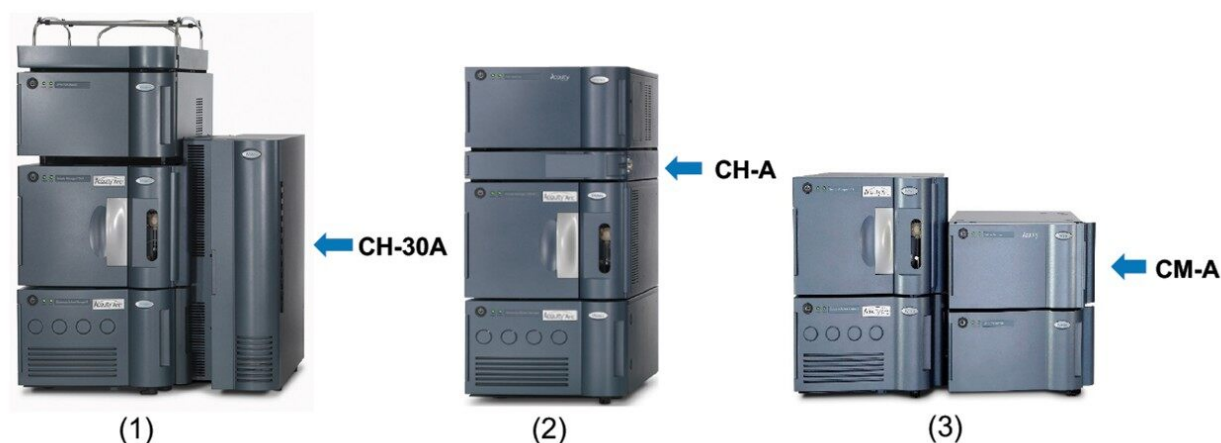


Figure 1. ACQUITY Arc System with different column compartment options: (1) CH-30A Column Heater; (2) CH-A Column Heater; and (3) CM-A Column Manager.

## Results and Discussion

The six sugar standards (fructose, galactose, glucose, sucrose, lactose, and maltose) and the stable isotope labeled internal standards (fructose- $^{13}\text{C}_6$ , glucose- $^{13}\text{C}_6$ , sucrose- $^{13}\text{C}_6$  and lactose- $^{13}\text{C}_6$ ) were injected in the ACQUITY Arc System that has been configured with each of the three options, the CH-30A, CH-A, and CM-A column compartment options. The same XBridge BEH Amide XP 2.5  $\mu\text{m}$ , 3.0 x 150 mm Column was used in these three configurations. As displayed in Figure 2, the chromatograms acquired in these three column compartment configurations were almost identical. The average retention time (RT), QDa Mass Detector response (relative to internal standards), and separation resolution (calculated by using peak width at half height of the peak) of sugar peaks for six replicated injections in three different column compartment configurations are shown in Table 1. The RT, response, and the resolution results obtained with the CH-A and the CM-A are very close to the results with the CH-30A column compartment. The resolution for the critical pair of galactose and glucose are all above 1.20 in the three column compartment configurations. These results demonstrate that the new column compartments, specifically the CH-A and the CM-A column options, perform equivalently to the CH-30A column compartment in the chromatographic separation of the sugar compounds. Other ACQUITY Arc System column compartments, such as the 30-cm CH/C or 30-cm CH, CM-30S, and CM-Aux, were not suitable for the sugar analysis and were not evaluated in this study.

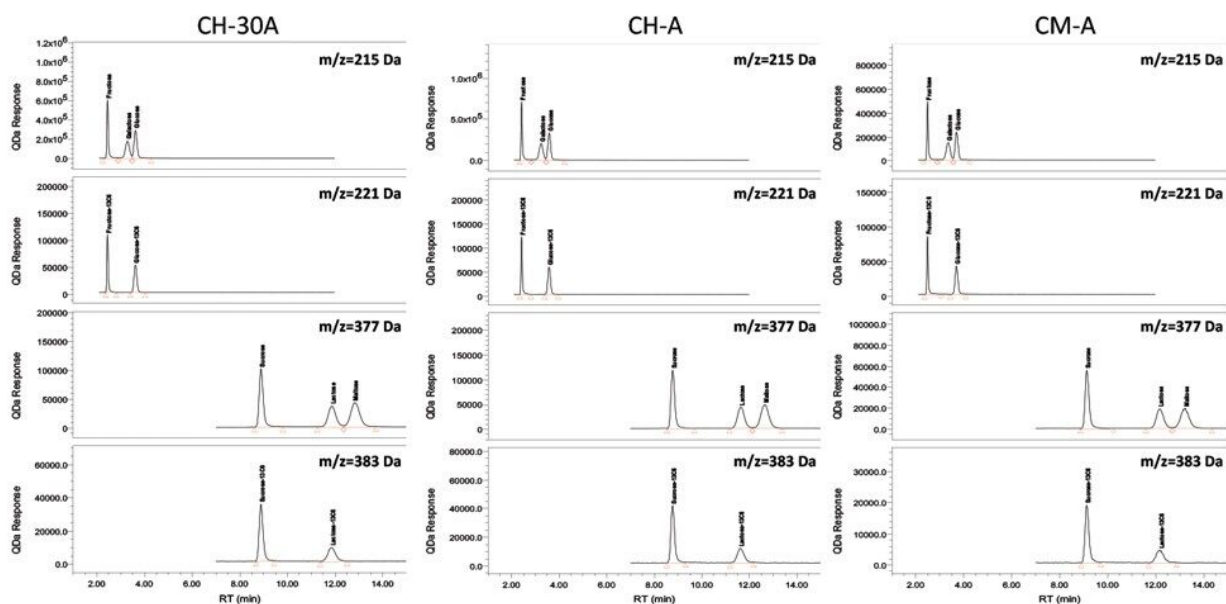


Figure 2. Chromatograms of sugar standards acquired on an ACQUITY Arc System with CH-30A, CH-A, and CM-A column compartments. The  $^{13}\text{C}$  stable isotope labeled sugars were used as internal standards.

	N. of Inj.	CH-30A			CH-A			CM-A		
		RT (min)	Response	Resolution	RT (min)	Response	Resolution	RT (min)	Response	Resolution
Fructose	6	2.46	5.81		2.42	5.86		2.51	6.08	
Galactose	6	3.31	3.44	3.86	3.23	3.52	4.00	3.37	3.60	4.08
Glucose	6	3.65	5.95	1.20	3.57	5.94	1.29	3.72	6.24	1.27
Sucrose	6	8.96	2.95		8.72	2.90		9.19	3.10	
Lactose	6	11.96	4.37	6.67	11.56	4.42	6.98	12.24	4.44	7.17
Maltose	6	12.95	5.76	1.56	12.56	5.61	1.74	13.30	5.06	1.78

Table 1. The average retention times, QDa responses, and separation resolutions of six standards in repeated measurements ( $n=6$ ) on the same XBridge BEH Amide XP Column and the same ACQUITY ARC System, but with different CH-30A, CH-A, and CM-A column compartments.

## Conclusion

The ACQUITY Arc System with the ACQUITY QDa Mass Detector provides a robust and reliable analytical system for the sugar analysis in foods. The ACQUITY Arc System CH-A Column Heater and the CM-A

Column Manager are equivalent to the ACQUITY Arc CH-30A Column Heater in terms of separation of sugar compounds, including the critical pair of galactose and glucose.

When lab space is a concern, the CH-A Column Heater allows a smaller footprint of the primary stack. The CM-A Column Manager accommodates columns for different applications or method development. These new column compartment options provide more flexibility to the ACQUITY Arc System and at the same time maintain the same separation performance in the sugar analysis.

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## References

1. Yang, J.; Rainville, P.; Liu, K.; and Pointer, B. Quantification of Mono and Disaccharides in Foods. Waters Corporation Application Note, 720006575EN, 2019.

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## Featured Products

[ACQUITY Arc System <https://www.waters.com/134844390>](https://www.waters.com/134844390)

[ACQUITY QDa Mass Detector <https://www.waters.com/134761404>](https://www.waters.com/134761404)

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