

### Improve a Waters Method for Aromatic Acids by Using an Agilent ZORBAX RRHD Column and an Agilent 1290 Infinity LC

### **Application Note**

Food Testing & Agriculture

### Abstract

A Waters method for the analysis of aromatic acids was transferred to an Agilent 1290 Infinity LC with an Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18 column, without additional method development. Comparing the Waters Acquity UPLC CSH C18 and RRHD Eclipse Plus C18 columns on the Waters Acquity UPLC system showed that the Agilent column generated 10% lower pressure, while improving peak shape, most notably for salicylic acid, which was 48% narrower and 89% more symmetrical with the Agilent column. Comparing the Waters Acquity UPLC and a 1290 Infinity LC with the RRHD column showed that the Agilent system generated 13% less pressure than the Waters system while producing 3 to 9% narrower peaks and improving resolution by 7 to 14%.

### Introduction

This application note demonstrates the complete transfer of a method in a Waters publication [1] to a RRHD Eclipse Plus C18 column with a 1290 Infinity LC. The method shows a gradient acetic acid/acetonitrile separation of 7 aromatic acids on a Waters Acquity UPLC CSH C18,  $2.1 \times 50$  mm,  $1.7 \mu$ m column with a Waters Acquity UPLC system [1]. Analysis of 6 of the compounds (2,4-dichlorophenoxyacetic acid was not available) was transferred stepwise to an RRHD Eclipse Plus C18,  $2.1 \times 50$  mm,  $1.8 \mu$ m column with a 1290 Infinity LC. Performance improvements were evident with the Agilent column and the Agilent system compared to their Waters counterparts.



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### **Materials and Methods**

LC method parameters were kept as consistent as possible. Table 1 shows the conditions. All chromatograms were analyzed in Agilent OpenLAB CDS ChemStation Edition Revision C.01.03. All data were collected within 1 week with supplies refrigerated or frozen, as necessary, to prevent degradation. Column and system performance were verified daily to ensure comparable results over the course of the experiment.

All aromatic acids shown in Figure 1, and glacial acetic acid, were purchased as powders from Sigma-Aldrich Corp. Acetonitrile was purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.



Figure 1. Aromatic acids.

	Waters Acquity UPLC System			Agilent 1290 Infinity LC		
Column	Waters (p/n 18	Acquity 6005296	UPLC CSH C18, 2.1 × 50 mm, 1.7 µm ), serial number 01133209515691	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 μm (p/n 959757-902), serial number USDAY06281, lot number B11188		
Mobile phase (A, B)	0.1% acetic acid, acetonitrile					
Gradient	Time	%B	Curve	Time	%В	
	Initial	2	Initial	0.00	2	
	5.00	60	6	5.00	60	
	5.50	60	6	5.50	60	
	5.51	2	6	5.51	2	
	6.00	2	6	6.00	2	
Injection	$4~\mu L$ of 0.01 mg/mL sample in water, partial sample loop with needle overfill			4 $\mu L$ of 0.01 mg/mL sample in water, ADVR on		
Detection	280 nm, 4.8 nm resolution, 20 points/s, fast filter time constant			Sig = 280,4 nm; ref = off, slit 4 nm, PW > 0.0031 min (0.063 s response time) 80 Hz		
Configuration	Acquity UPLC System (BSM, SM) with Acquity UPLC PDA			G4220A, G4226A, G1316C, G4212A with Ultra Low Dispersion Kit (5067-5189), Ultra Low Dispersion Max-Light Cartridge Flow Cell, $V(\sigma) = 0.6~\mu$ L (G4212-60038), LC System Rack (5001-3726)		
Flow rate	0.6 mL/min					
Column temperature	30 °C					
Sample preparation	Individual standards: each prepared in water:acetonitrile (5:1) Protocatechuic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, salicylic acid 2,4-Dichlorophenoxyacetic acid was not available					
	Stock solution: 1 mL each of individual standards (6 mL total), 0.278 mg/mL each					
	Sample: 0.5 mL stock solution + 9.5 mL water, 0.0139 mg/mL each					

Table 1. LC method parameters for the analysis of aromatic acids on a Waters Acquity UPLC System and an Agilent 1290 Infinity LC.

Sample concentration 1.67 mg/mL

#### **Results and Discussion**

0.05

0

'n

0.5

### Reproducing a Waters method for the analysis of aromatic acids

The original analysis of aromatic acids was reproduced (top chromatogram in Figure 2) on a Waters Acquity UPLC CSH C18 column with a Waters Acquity UPLC System. Retention times and peak shapes appeared very similar to those published [1], with the exception of protocatechuic acid (peak 1), which appeared to tail more in Figure 2 than in the original publication. Peak heights were slightly increased in Figure 2 compared to the original due to a slightly higher sample concentration.

# Replacing the Waters Acquity UPLC column with a RRHD Eclipse Plus C18 column

The Waters column was then replaced with a same dimension RRHD Eclipse Plus C18 on the same Waters Acquity UPLC system (bottom chromatogram in Figure 2), running the same instrument method with the same sample and mobile phases. Improvements to all peaks are apparent, including peak width, peak height, and peak symmetry, while system pressure was reduced by 10%. Most notably, the salicylic acid peak (peak 6) on the RRHD Eclipse Plus C18 column was 48% narrower and 89% more symmetrical than on the CSH C18 column (symWaters = 0.36, symAgilent = 0.68). Minimum resolution for this aromatic acids analysis was also better with the Agilent column, improving minimum resolution by 10%, from 2.76 to 3.03.



Figure 2. Original analysis of aromatic acids on a Waters Acquity UPLC CSH C18 column (top) and an Agilent ZORBAX RRHD Eclipse Plus C18 column (bottom), both on a Waters Acquity UPLC system. The Waters column performance is similar to published results [1], while peak shape improvements are evident with the Agilent column.

2.5

3

3.5

'n

4.5

min

2

1.5

# Transferring from the Waters Acquity UPLC system to a 1290 Infinity LC

Next, the RRHD Eclipse Plus C18 column was transferred to a 1290 Infinity LC (in its default configuration, as shipped from Agilent). LC method parameters were kept as consistent as possible, while the same mobile phases, sample, and column were used (top chromatogram in Figure 3). All peaks were wider, shorter, and eluted later on the Agilent system as compared to the Waters system. However, the Agilent system generated 17% less pressure than the Waters system (426 bar versus 512 bar), probably due to increased extra-column and delay volumes in the Agilent LC system.

# Optimizing extra-column and delay volumes of the 1290 Infinity LC

Improvements were easily made to the 1290 Infinity LC to reduce extra-column and delay volumes. First, the Ultra Low Dispersion (ULD) kit replaced the standard 0.12 mm id capillaries with 0.075 mm id capillaries. An Ultra Low Dispersion Max-Light Cartridge Flow Cell was also installed, and an Agilent LC System Rack was used to arrange the modules such that the shortest possible capillaries could be used. The result was a ULD 1290 Infinity LC with 64% less extra-column volume than the default system (4 versus 11  $\mu$ L) [2]. All peaks (middle chromatogram in Figure 3) were noticeably taller, as well as 11 to 16% narrower. Because of



Figure 3. Analysis of aromatic acids with an Agilent ZORBAX RRHD Eclipse Plus C18 column on an Agilent 1290 Infinity LC in default configuration (top), ultra low-dispersion configuration (middle), and reduced delay volume configuration (bottom). Reducing extra-column volume sharpened peaks and improved resolution, while minimizing delay volume reduced gradient retention and further improved peak shape.

the improved peak width, resolution was also improved by 12 to 24%, with the critical pair increased by 24%, from 2.96 to 3.67 for peaks 4 and 5 (vanillic and syringic acid).

Additionally, a modification to the LC method parameters can reduce the system delay volume. The automatic delay volume reduction (ADVR) function built into the software for the Agilent 1290 Infinity Autosampler effectively reduces system delay volume by 63% without further hardware adjustments (123 to 49  $\mu$ L). The ADVR changes the typical flow path through the autosampler, where the needle is continually flushed, to a bypass flow path. Here, the injection valve switches from main pass to bypass after the injection has been made and sample has been flushed onto the column. This removes the needle, metering device, and associated capillaries from the flow path of the gradient, allowing the mobile phase to reach the head of the column more quickly [3,4]. While very fast gradients are affected most by the system delay volume, improvements are still evident with this more classic gradient analysis of aromatic acids (bottom chromatogram in Figure 3). Retention times for all peaks

decreased by 6 to 9%, with early eluting peaks more affected than later eluting peaks. Peak widths were also slightly reduced (2 to 7%), most likely due to the shortened retention times.

Figure 4 illustrates the use of the ULD kit and flow cell as well as the ADVR function with a 1290 Infinity LC, and it shows the volumetric changes that result with respect to extra column and system delay volumes. According to the published instrument specifications, a standard Waters Acquity UPLC system has a delay volume of approximately 120 µL, while the system band spread (extra-column volume) is unreported [5]. The instrument specifications for the Waters low dispersion Acquity UPLC I-Class is reported to have a system delay volume of about 100 µL, while the system band spread is quoted at 6 to 9  $\mu$ L [6]. While the standard Waters Acquity UPLC system is used in this experiment, the volumetric data in Figure 4 indicate that this optimized 1290 Infinity LC will have lower extra-column and delay volumes (4 and 49 µL, respectively) than the optimized Waters Acquity UPLC I-Class system.<sup>1</sup>



Figure 4. An Agilent 1290 Infinity LC in default configuration (left), ultra low-dispersion configuration (middle), and reduceddelay-volume configuration (right). The volumetric impact of these system changes on extra-column and delay volumes are shown in the table beneath the diagram.

<sup>&</sup>lt;sup>1</sup> These volumes represent an Agilent 1290 Infinity LC customized for this particular analysis. Further modifications to the LC system volumes are possible depending on instrument requirements for other analyses.

The optimized 1290 Infinity LC (bottom chromatogram in Figure 3) was compared with the standard Waters Acquity UPLC (bottom chromatogram in Figure 2) in the analysis of aromatic acids, using an RRHD Eclipse Plus C18 column. The lower extra-column and delay volumes in the Agilent system yielded 1 to 5% shorter retention times, with 3 to 9% narrower peaks. Resolution improved by 7 to 14%, with the critical pair improved by 14% from 3.03 to 3.45. While the optimizations to the Agilent system came at the cost of increased system pressure (optimized pressure is 4% higher than the default system), the pressure generated by the 1290 Infinity LC was still substantially less (13%, 443 versus 512 bar) than the pressure generated by the Waters LC, allowing for more flexibility to increase flow rate and column length. Further flexibility is afforded by the 1290 Infinity LC, as the optimized setup is completely removable, and the original capillaries can easily be partially or fully reinstalled for additional pressure flexibility should an analysis require it.

### Comparing the newly transferred Agilent analysis to the Waters analysis

A final assessment of the original application as performed on the Waters Acquity UPLC CSH C18 column with a Waters Acquity UPLC system, compared to the same analysis on the RRHD Eclipse Plus C18 column with a 1290 Infinity LC, demonstrated the complete method transfer (Figure 5). The Agilent solution for aromatic acids generated 22% less pressure (566 versus 443 bar) than the Waters solution. The Agilent solution also delivered 6 to 52% narrower peaks that



Figure 5. The original analysis of aromatic acids with a Waters column on a Waters LC system (from Figure 2) (top), and the same analysis improved with an Agilent ZORBAX RRHD Eclipse Plus C18 column on an Agilent 1290 Infinity LC (from Figure 3) (bottom). The Waters solution was improved by transferring this analysis to an Agilent solution; peaks were all substantially sharper and more symmetrical. Minimum resolution was also improved.

were 6 to 114% more symmetrical; the salicylic acid peak (peak 6) performance was most improved in both cases with symmetry improving from 0.36 to 0.77. Resolution was also improved by more than 25% for all peaks with the Agilent system and column solution.

### Conclusions

An analysis of aromatic acids demonstrated in a Waters publication was easily transferred to an Agilent ZORBAX RRHD Eclipse Plus C18 column with an Agilent 1290 Infinity LC. Both the Agilent column and system provided performance improvements over the Waters equivalents without any additional method development.

#### References

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