Nota de aplicación

Performance Comparison of a Gradient Method Using a Microbore (1 mm Internal Diameter) column on ACQUITY UPLC and Competitive UHPLC Instrumentation

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Abstract

In this application note a method for diclazuril based of the USP monograph and scaled to a 1 mm I.D. column is used to demonstrate the extra column dispersion and the effect it has on the resulting chromatography.

In order to achieve high quality separations using 1.0 mm I.D. columns, the user must be cognizant of the LC instrument characteristics and the potential negative effects it may have on the resultant chromatography. In order to maximize resolution between peaks, it is important to have the lowest possible dispersion in the instrument. The ultra-low dispersion of the ACQUITY UPLC I-Class System provides exceptional chromatographic performance when running microbore columns.

Benefits

Improved chromatographic performance with a lower dispersion instrument when running 1 mm I.D. columns.

Introduction

In reversed-phase liquid chromatography, there is an important link between instrument characteristics and the chromatography generated. The flow path of any chromatographic system consists of many components that will contribute to the distortion and broadening of chromatographic peaks, which is often referred to as band spread or dispersion. This includes but is not limited to the injector, the flow path tubing, the column, flow cells and any additional valves along the flow path. Dispersion can be further broken down into column dispersion, or that arising from the chromatographic column, and extra column dispersion, which refers to the contribution from the liquid chromatography (LC) instrument components. This application note will specifically focus on the extra column dispersion for two LC instruments, and the effect it has on the resulting chromatography.

In addition to understanding what causes dispersion and the effects it has on chromatography, it is also important to match the internal diameter (I.D.) of a column to the system dispersion in order to produce optimal chromatography. Using microbore columns, or those with internal diameters of 1 mm or less, offers a number of benefits to the user, including decreased solvent consumption, decreased sample consumption and decreased waste production. However when the column diameter is decreased peak volumes are also decreased. It is known that extra-column dispersion effects are increased as peak volumes are decreased.[1] Thus, if the instrument design does not provide low extracolumn dispersion, the resulting chromatography will not realize the benefits of decreasing column I.D. Some potential issues are peak broadening, loss of resolution, and decreased peak capacity. To see chromatographic gains in these areas, it is important that both the analytical column and LC instrument design have been optimized. In this application note a method for diclazuril based of the USP monograph[2] and scaled to a 1 mm I.D. column is used to demonstrate these concepts.

Experimental

Sample description

Diclazuril System Suitability Mixture RS was purchased from United States Pharmacopeia. Sample was dissolved and diluted in dimethylformamide (DMF) to a final concentration of 0.5 mg/mL.

LC conditions

LC systems:	Competitor UHPLC System ACQUITY UPLC I-Class System with CH-A
Detection:	Competitor DAD for UHPLC System and ACQUITY UPLC PDA Detector
Sample:	Diclazuril System Suitability Mixture RS (USP catalog number 1188571)
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 1.0 x 50 mm
Column temperature:	35°C
Mobile phase A:	85:15 10 mm Ammonium formate, pH = 4: Acetonitrile

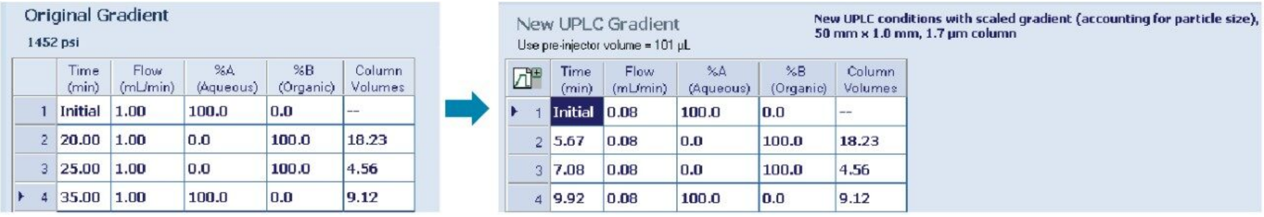
Mobile phase B:	85:15 Acetonitrile: 10 mm Ammonium formate, pH = 4
Flow rate:	0.084 mL/min
Injection volume:	0.5 µL
Wavelength:	230 nm
Collection rate:	20Hz
Needle wash:	90:10 Methanol/Water
Seal wash:	80:20 Water/Methanol
Chromatography software:	Empower 3 FR2, Chromeleon 7.2

Gradient

Time	%A	%B	Curve
0.0	100	0	-
5.67	0	100	6
7.08	0	100	1
8.50	100	0	1

Results and Discussion

The United States Pharmacopeia (USP) method for the analysis of diclazuril and related substances was scaled from the original HPLC configuration, 4.6 x 100 mm 3 µm column, to a micro-bore 1 x 50 mm 1.7 µm column using the ACQUITY UPLC Columns Calculator. The flow rate was scaled accounting for particle size and decreased from 1.00 mL/min in the original method to 0.084 mL/min in the updated method. A volume of 0.5 µL of sample was injected onto the micro-bore column. The ACQUITY UPLC Columns Calculator also generated the updated LC method table which maintains the gradient slope when scaling from the original HPLC column to the updated microbore column (Figure 1). The method was then analyzed on both an ACQUITY UPLC I-Class System, as well as a competitive binary UHPLC system (Figure 2). Both systems were configured according to manufacturer's guidelines or recommendations.



Original Gradient

1452 psi

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Organic)	Column Volumes
1	Initial	1.00	100.0	0.0	--
2	20.00	1.00	0.0	100.0	18.23
3	25.00	1.00	0.0	100.0	4.56
4	35.00	1.00	100.0	0.0	9.12

New UPLC Gradient

Use pre-injector volume = 101 µL

New UPLC conditions with scaled gradient (accounting for particle size), 50 mm x 1.0 mm, 1.7 µm column

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Organic)	Column Volumes
1	Initial	0.08	100.0	0.0	--
2	5.67	0.08	0.0	100.0	18.23
3	7.08	0.08	0.0	100.0	4.56
4	9.92	0.08	100.0	0.0	9.12

Figure 1. Example of a scaled method, accounting for particle size, generated using the ACQUITY UPLC Columns Calculator.

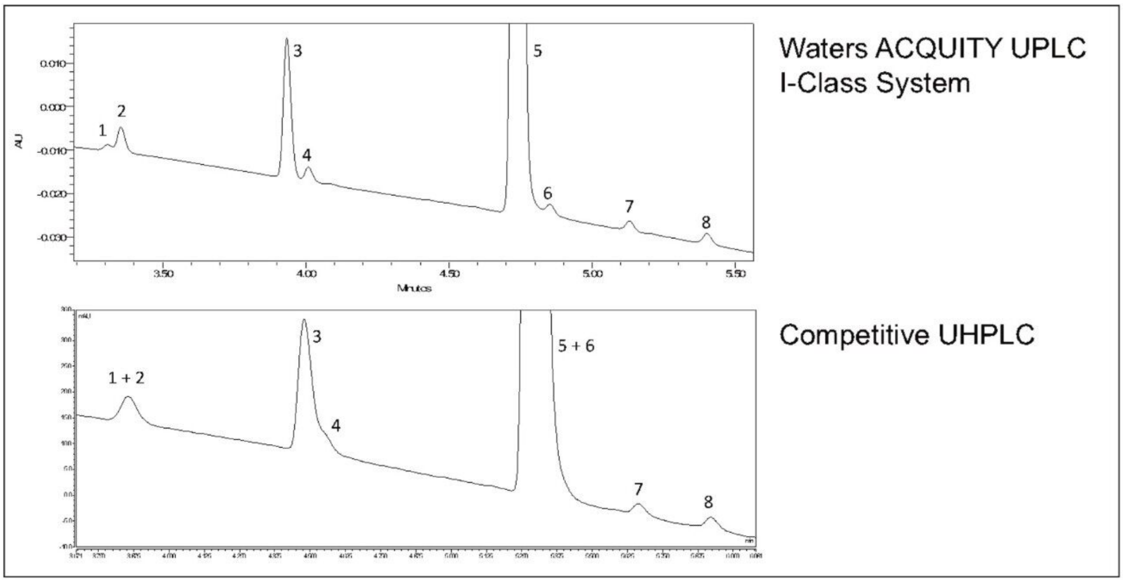


Figure 2. Chromatographic comparison of diclazuril system suitability mixture run on an ACQUITY UPLC I-Class (top) and competitive UHPLC system (bottom).

Differences in chromatographic performance are evident in this separation. The chromatogram acquired on the UPLC instrument shows the presence of additional peaks that could not be resolved using the UHPLC instrument. In fact, peaks 1 and 2, and peaks 5 and 6 are clearly resolved in the UPLC chromatogram, but could be mistaken for a single peak in the UHPLC chromatogram. This would be problematic for a number of reasons; ultimately leading to erroneous results.

Since both chromatograms were generated using the identical sample, instrument conditions, and chromatographic column, the differences arising in the chromatographic performance are the result of the characteristics of the specific systems. As stated in the introduction, extra-column dispersion is an important characteristic of an LC system because of its effect on the resulting chromatography. Increased extra-column dispersion results in broadening of the chromatographic peaks. When measured under identical conditions, the UHPLC system had a measured extra-column dispersion volume approximately double the measured extra-column dispersion volume of the ACQUITY UPLC I-Class System. In this example, the larger system dispersion and its impact upon the chromatography ultimately led to peak co-elution. This is undesirable for a number of reasons, including but not limited to errors in peak identification and quantitation.

Using micro-bore columns, or those with internal diameters of 1 mm or less, offers a number of benefits to the user, including decreased solvent consumption, decreased sample consumption and decreased waste production. However, as the internal diameter of the column is decreased, the negative effect from system dispersion is magnified.[1] The ultra-low dispersion design of the ACQUITY UPLC I-Class provides users with the ability to run 1 mm I.D. columns without needing an instrument system specialized for low flow applications. The increased dispersion of the competitive UHPLC system may not provide users with the ability to run 1 mm I.D. columns because of the decrease in chromatographic performance.

Conclusion

In order to achieve high quality separations using 1.0 mm I.D. columns, the user must be cognizant of the LC instrument characteristics and the potential negative effects it may have on the resultant chromatography. In order to maximize resolution between peaks, it is important to have the lowest possible dispersion in the instrument. This is especially true for applications run on micro-bore columns; as the effects of band broadening will be exacerbated. The ultra-low dispersion of the ACQUITY UPLC I-Class System provides exceptional chromatographic performance when running microbore columns.

References

1. Snyder, Lloyd R., Kirkland, Joseph J., Dolan, John W., *Introduction to Liquid Chromatography, 3rd Edition*. Hoboken: Wiley and Sons, 2010. Print.
2. Official Monographs, USP 37 NF32 S2. *United States Pharmacopeia and National Formulary* (USP 37-NF 32 S2) Baltimore, MD: United Book Press, Inc.; 2014. p. 4968.

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