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ULDC – Effects of extra column volume and extra column dispersion

Kristin Folmert, Carsten Losch, Kate Monks; applications@knauer.net

KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38,
14163 Berlin; www.knauer.net

SUMMARY

Ultra **L**ow **D**ispersion **C**hromatography, short ULDC, makes use of the reduction of the system volume to enhance the separation by improvement of e.g., peak shape/width, resolution, and signal-to-noise ratio. A comparison of different system volumes was performed to show the effect of extra column dispersion on system performance. Using the new KNAUER AZURA® 862 ULDC chromatography systems makes it easy enhance the performance compared to standard HPLC systems.

INTRODUCTION

When considering the optimization of a HPLC system often the pre- and post-column volume, and their impact on the performance, are often underestimated. To optimize dispersion volume and band broadening, various aspects of a system should be considered [1], for example the volume of the injector, capillaries, measuring cell and column volume itself should be reduced when optimizing a system. Reducing the column size to smaller dimensions, e.g., 50 x 2 mm ID, is also beneficial because it shortens the runtime and can therefore enable high throughput analysis. To support the smaller volumes required for optimization the HPLC system must be able to withstand the resulting higher pressure. With the AZURA 862 ULDC system, all applications up to 862 bar can be carried out, allowing UHPLC to be carried out on a standard system – this makes dedicated UHPLC systems obsolete.

Additional Information

In this application note we investigate how reducing the extra column volume in the AZURA 862 results in improved separations.

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SAMPLE PREPARATION

A mixed standard of eight compounds was used. The stock solution was prepared with 50 % acetonitrile. This stock solution was diluted in a ratio 1:100 with 25 % acetonitrile.

Tab. 1 Compounds and concentrations of stock solution and dilution

Substance	Stock solution (mg/ml)	Dilution 1:100 (mg/ml)
Uracil	0.50	0.005
Resorcinol	5.00	0.050
Aniline	2.30	0.023
2-Nitrobenzyl alcohol	1.00	0.010
4-Nitrophenol	2.00	0.020
N,N-Dimethylaniline	0.40	0.004
Ethyl benzoate	2.40	0.024
Toluene	7.80	0.078

RESULTS

Diffusion occurs in the internal volume of the injection system, as well as the detector, tubings, and any other devices present in the flow path. Collectively, the volume of these components is known as extra column volume (ECV). ECV was determined with an isocratic method by injecting a 140 µg/ml caffeine solution with a low volume coupling/connector instead of a column (n=10). The used method is documented in **Tab. 4** in the Materials and Methods part.

For the standard HPLC configuration the ECV was determined as 24 µL and could be reduced to 8.50 µL applying ULDC settings. Comparing the standard HPLC configuration to ULDC dimensions a significant decrease of about 65 % for ECV was achieved. **Fig. 1** shows the comparison of theoretically calculated ECV and measured ECV. The measured values are in same range but slightly higher than the calculated volumes. This can be caused by diffusion effects or also from e.g., dead volume of couplings, valves, or fittings.

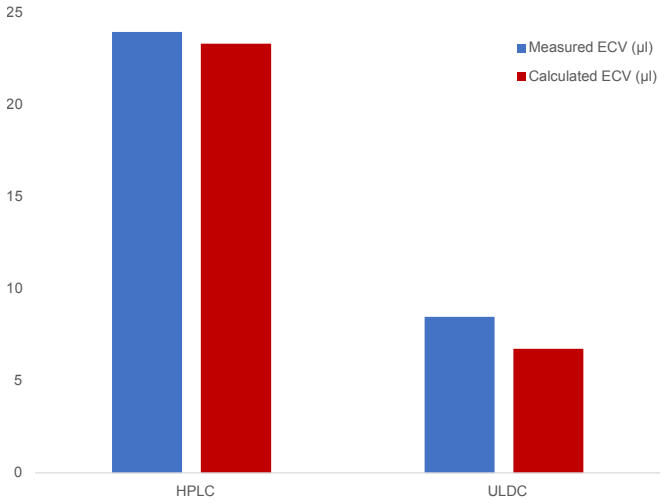

Fig. 1 Comparison of calculated and measured Extra Column Volume (ECV)

After determination of ECV the diluted mixed standard **(Tab. 1)** was measured. The used method is documented in **Tab. 5** the Materials and Methods part. For evaluation of the effects caused by reduction of system volume, following parameters were investigated: peak height, peak area, peak width, theoretical plates, resolution, and signal to noise ratio. **Fig. 2** exemplary shows the effect on peak width, and peak height when applying the different system configurations using a column in a dimension 50 x 2.1 mm ID and 1.9 µm particle size.

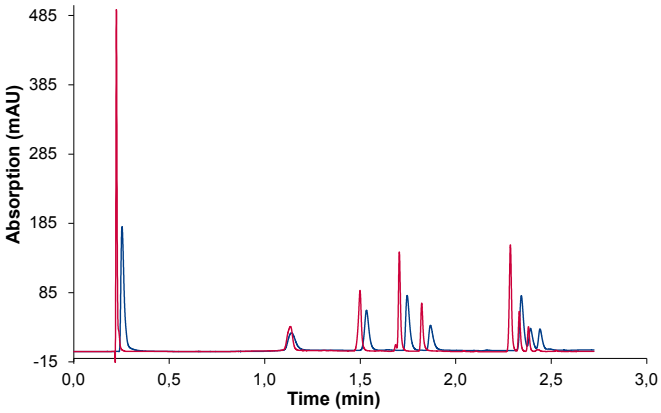

Fig. 2 Comparison of the peak height and width, blue – HPLC, red – ULDC

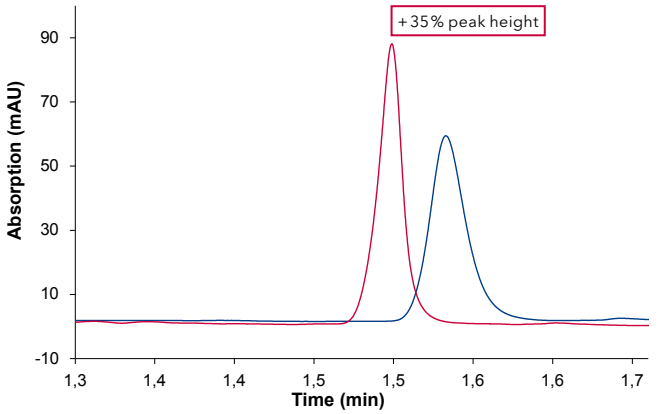

Fig. 2A Enhanced view of figure 2 (peak 3 – aniline), blue – HPLC, red – ULDC

From HPLC to ULDC a raise of signal intensity/peak height of 35 % was achieved. The S/N ratio was enhanced in a range from 56 % for toluene up to 156 % for N,N-dimethylaniline. The peak width$_{0.5}$ was reduced by 0.72 seconds.

Tab. 2 Comparison of peak area, peak width $w_{0.5}$ peak height and S/N ratio for gradient elution

Analyte	HPLC				ULDC			
	Area (mAU*s)	Peak width$_{0.5}$ (s)	Peak height (mAU)	S/N ratio	Area (mAU*s)	Peak width$_{0.5}$ (s)	Peak height (mAU)	S/N ratio
Uracil	3.834	1.08	180.27	3338	3.235	0.30	489.04	11643
Resorcinol	1.160	2.64	24.98	463	1.016	1.80	33.67	802
Aniline	1.604	1.50	57.60	1067	1.600	0.96	87.37	2080
2-Nitrobenzyl alcohol	1.896	1.32	78.85	1460	1.766	0.60	137.47	3273
4-Nitrophenol	1.023	1.44	36.10	669	0.990	0.72	70.10	1669
N,N-Dimethylaniline	1.854	1.32	77.91	1443	2.164	0.72	155.20	3695
Ethyl benzoate	0.790	1.32	32.20	596	0.678	0.66	56.91	1355
Toluene	0.772	1.32	31,413	582	0.495	0.66	38.22	911

The reduction of system volumes naturally also results in an increase in pressure. For gradient elution with a flow rate of 0.7 ml/min a maximum pressure of about 510 bar was measured using ULDC configuration. Nevertheless, with the AZURA 862 systems this is not an issue and enough reserves for even higher flow rates are available.

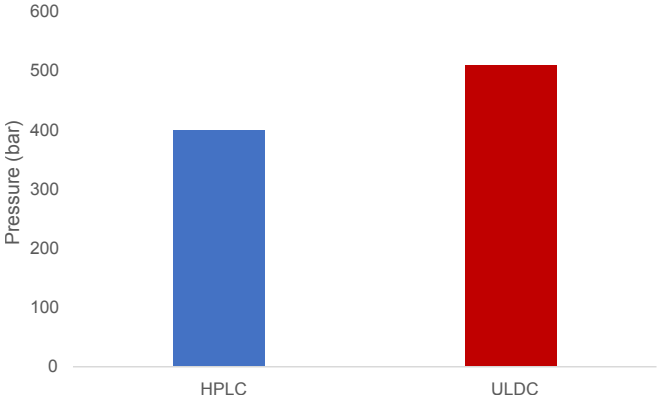

Fig. 3 Maximum pressure values

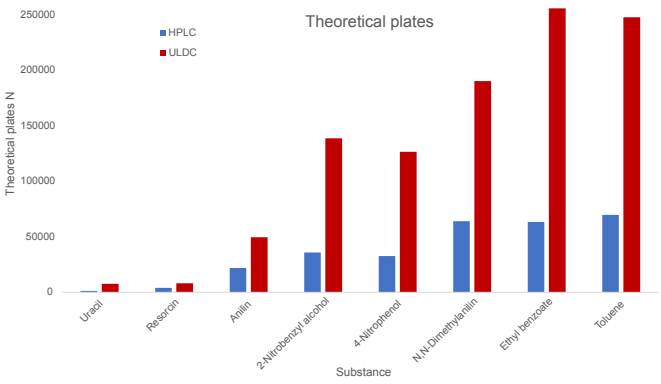

Fig. 4 Comparison of theoretical plates

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In **Fig. 4** the increase of the theoretical plates is shown, **Fig. 5** displays the change of peak width at a level of 5σ (4.40 %) of peak height.

The theoretical plate number is used as a measure for column efficiency. Columns with high plate numbers are considered to be more efficient. So, the increase of plates is beneficial to resolve narrow peaks.

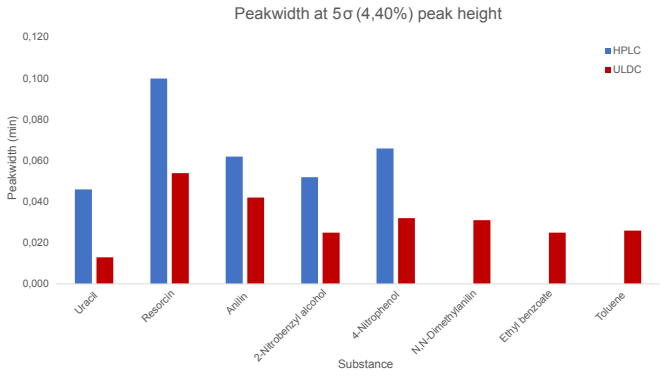

Fig. 5 Comparison of peak width at level 5 σ

As seen in **Fig. 5**, the peak width at 5σ for the last three peaks could only be determined using the ULDC configuration. This was caused due to the improved resolution of these critical peaks when decreasing the system volume. Smaller peak widths and therefore a better resolution and signal to noise value lead to more stable methods and easier quantification of analytes.

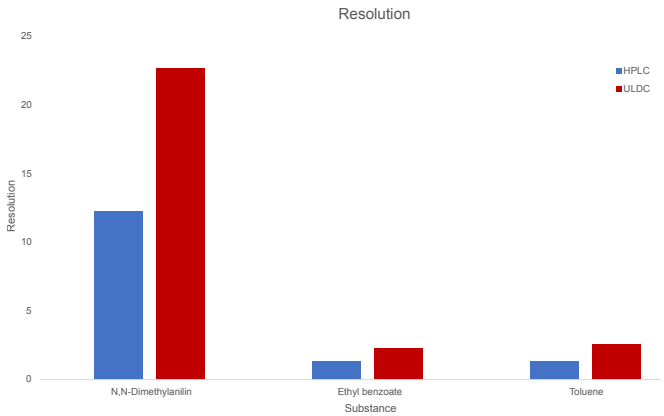

Fig. 6 Improvement of resolution for critical peaks

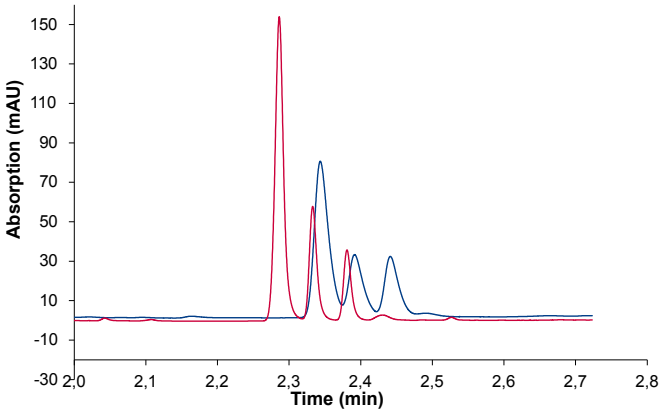

Fig. 6A Improvement of resolution for critical peaks, blue – HPLC, red – ULDC

Comparing the resolution values from HPLC to ULDC configuration, finally a baseline separation was achieved.

Tab. 3 Improvement of resolution for critical peaks

Peak	Analyte	HPLC	ULDC	Increase (%)
6	N,N-Dimethylanilin	12.28	22.72	54.05
7	Ethyl benzoate	1.32	2.33	56.65
8	Toluene	1.33	2.56	51.91

CONCLUSION

With this work it was shown that it is possible to significantly enhance the performance when decreasing the ECV in the system. Even with the resulting backpressure, the AZURA ULDC system is able to adapt the methods up to 862 bar. Not only the ECV decreases, also linked to that is the decrease of the peak width. Therefore, if the peak width is reduced while the area remains the same the peak height rises, resulting in a better signal to noise ratio and enhanced sensitivity.

MATERIAL AND METHODS

System configuration

Instrument	Description	Article No.
System	AZURA 862 ULDC HPG System DAD 2.1L	A46026
Capillary	AZURA 862 ULDC Kit	A9990
Column	Supelco Titan C18, 2.1x 50 mm, 1.9 µm	05BD181TIE
Software	Chromeleon™ 7.2	—
Software	Chromeleon™ 7.2 Drivers	A1783-2

Tab. 4 Extra column volume method

Column temperature	25 °C		
Injection volume	1 µl		
Injection mode	Partial Loop		
Detection	273 nm		
Data rate	100 Hz		
Eluent A	Water		
Eluent B	Acetonitrile		
Flow rate	0.5 ml/min		
Pump program	Time (min)	(A) (%)	(B) (%)
	0	25	75
	2	25	75

Tab. 5 Gradient method for a 50x2.1 mm,1.9 µm C18 phase, a 10 mm, 2 µl measuring cell

Column temperature	30 °C		
Injection volume	5 µl		
Injection mode	Full Loop		
Detection	254 nm		
Data rate	100 Hz		
Eluent A	Water		
Eluent B	Acetonitrile		
Flow rate	0.7 ml/min		
Pump program	Time (min)	(A) (%)	(B) (%)
	0	95	5
	0.5	95	5
	3	0	100
	3.25	0	100
	3.5	95	5
	6	95	5

REFERENCES

[1] J.P Grinias, B. Bunner, M. Gilar, J. W. Jorgenson; Measurement and Modeling of Extra-Column Effects Due to Injection and Connections in Capillary Liquid Chromatography. Chromatography 2015

[2] A. Schultze-Jena, M.A. Boon, P.J.Th. Bussmann, A.E.M. Janssen, A. van der Padt; The counterintuitive role of extra-column volume in the determination of column efficiency and scaling of chromatographic processes. Journal of Chromatography A, 2017

[3] P. Hong, P.R. McConvill; Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer? Waters White paper, 2018

[4] Chromeleon™ 7.2

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FORMULAS

Extra column volume ECV (2,3)

$$ECV = Flow\ \left[\frac{ml}{min}\right] * Start\ time\ of\ Caffeine\ peak\ [min]$$

Theoretical plates EP (4)

$$TP = 5.54 * \left(\frac{t_R}{W_{50\%}}\right)^2$$

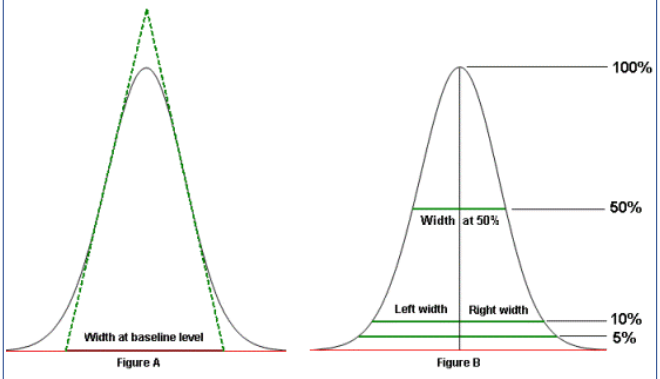
TP	Number of theoretical plates
t_R	Retention time
$W_{50\%}$	Peak width at 50 % height (half width)

Resolution EP (4)

$$R = 1.18 * \left|\frac{t_{RefPeak} - t_R}{W_{50\%,RefPeak} + W_{50\%,R}}\right|$$

t_R	Retention time of the current peak
$t_{RefPeak}$	Retention time of the reference peak for the resolution (By default, the reference peak is the peak after the current peak. However, you can select either the previous peak or a fixed peak as the reference peak.)
$W_{50\%,R}$ $W_{50\%,RefPeak}$	Widths of the two peaks at 50 % of the peak height

Peak width (4)



Option	Description
Compute Width at ...	
the baseline level	Click to calculate the width at baseline level (default).
5 % height over the baseline	Click to calculate the width at 5 % of the peak height.
10 % height over the baseline	Click to calculate the width at 10 % of the peak height.
50 % height over the baseline	Click to calculate the width at 50 % of the peak height.
Custom	If you want to calculate the width at a different height, click **Custom**. Type or select the height over the baseline in the box.

Signal to noise ratio (4)

According to the Peak to Peak method the Signal-to-Noise ratio is calculated as follows:

$$\frac{S}{N} = 2 * \frac{Peak\ Height}{Noise}$$

The value for Peak Height always corresponds to the related value of the peak variable *Height*.