Science Together

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Performance evaluation of KNAUER pulse dampers in comparison to competitive dampers

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SUMMARY

The KNAUER pulse dampers combine high damping performance with reliable membrane-free assembly and easy implementation into the HPLC system. Especially when working close to the limit of detection, they can significantly improve the detection of trace components by reducing noise of the baseline.

INTRODUCTION

Serial pumps like piston pumps are the standard solution for eluent delivery in HPLC systems. Although they are very reliable and require low-maintenance, residual pulsation cannot be fully prevented. Some applications such as trace component analysis are very sensitive to the residual pulsation of the pump. In these cases, addition of a pulse damper to the system improves the baseline and minimizes pressure impulses.

In this study, pulse dampers from two different competitors were compared with two prototypes of KNAUER pulse dampers.

Additional Information

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RESULTS

Damping efficiency

At some point during method development, the limit of detection (LOD) and the limit of quantification (LOQ) of an analyte need to be determined. To be able to quantify as low concentrations as possible, the baseline noise needs to be low. Consequently, a high damping efficiency of the pulse damper helps minimizing the noise and improves the signal-to-noise ratio.

The KNAUER pulse damper (high volume version) was compared to two dampers from competitors with regards to the damping efficiency and the dwell volume. **Fig.1** shows that the KNAUER pulse damper works with a damping efficiency of 95 % at 680 bar with a flow of 0.5 mL/min, the dampers of competitors 1 and 2 work with 90 % and 33 %, respectively.

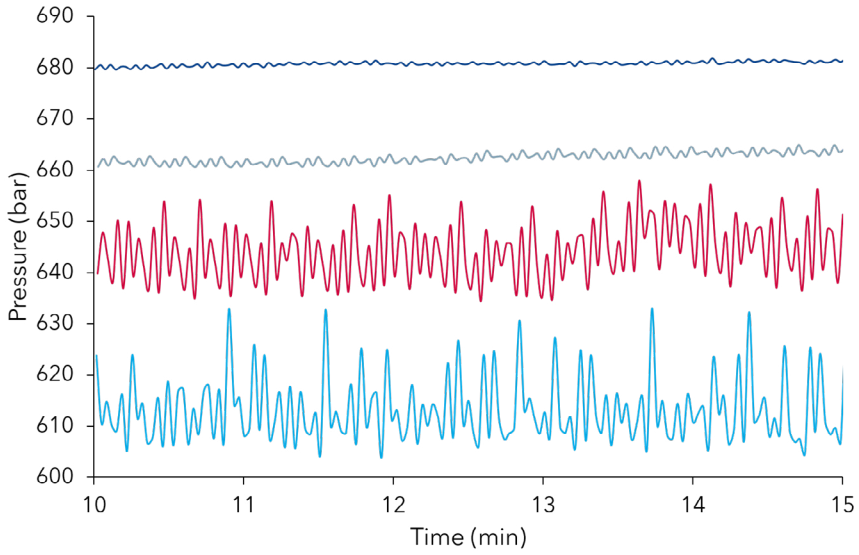

Fig. 1 Comparison of the KNAUER pulse damper (high volume version) with two competitor dampers and a configuration without pulse damper. The pressure trace was recorded with an AZURA P 6.1L pump with water at a flow rate of 0.5 mL/min; dark blue - KNAUER pulse damper, grey - competitor 1, red - competitor 2, light blue - no pulse damper

Dwell volume

To evaluate the additional dwell volume on the performance of the HPLC system the dwell volume of the different dampers was compared and a gradient test was performed. The dwell volume of every pulse damper was determined according to the method by Dolan, 2018 [1] **(Tab.1)**. The signals with the KNAUER pulse dampers reach a full plateau before the end of every gradient step, while the signals with the competitor dampers do not reach the same signal height **(Fig.2)**. These dampers appear to both have areas with insufficient perfusion and therefore would need a longer gradient program to reach the gradient steps. The KNAUER pulse dampers feature a flow path geometry designed for clean flush out and hence show a very effective mixing performance.

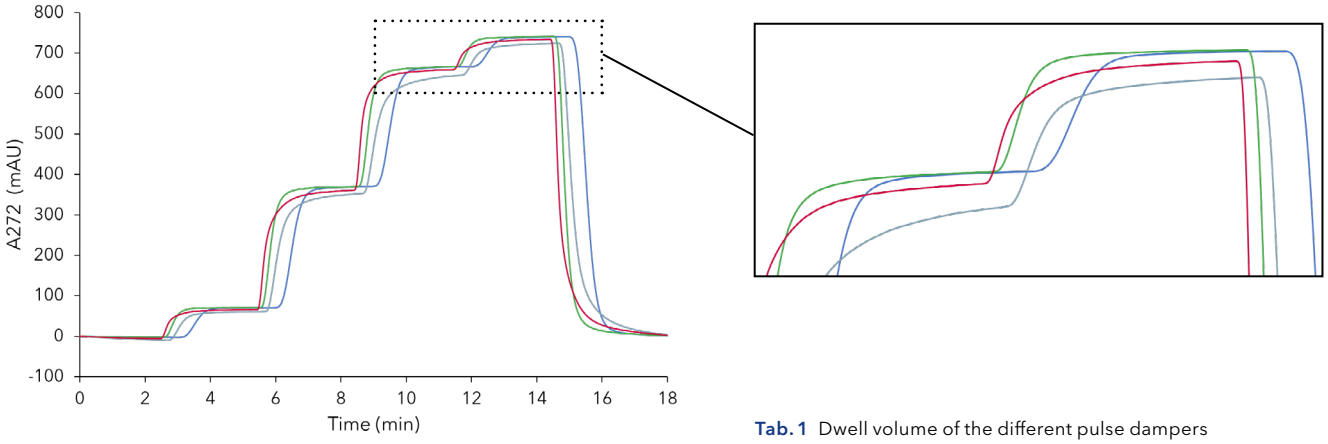

Fig. 2 Gradient test with the KNAUER pulse dampers (high (dark blue) and low (green) volume) and dampers of competitor 1 (grey) and 2 (red), mobile phase A: water, mobile phase B: water with caffeine as tracer (0.015 g/L); zoom in for more detailed view of gradient test

Tab. 1 Dwell volume of the different pulse dampers

Pulse damper	Dwell volume (µL)
KNAUER high volume (dark blue)	1465
KNAUER low volume (green)	625
Competitor 1 (grey)	935
Competitor 2 (red)	480

Performance Verification

The addition of a pulse damper to an existing gradient HPLC system introduces an additional volume after gradient formation, which results in a shift of retention time and may cause peak broadening. To investigate the influence of the two KNAUER pulse damper versions on these factors, a performance verification was carried out **(Fig. 3)**.

Signal to noise ratio was found to be improved by 50 % when using the high volume pulse damper and 30 % when the low volume version was used. A retention time shift of 8-12 seconds (low volume) and 17-23 seconds (high volume) was observed while peak height was reduced by 2.5-3.5 % (low volume) and 2.2-6.6 % (high volume).

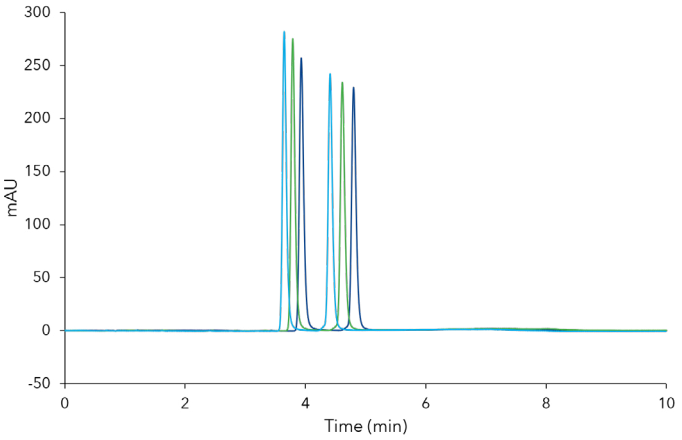

Fig. 3 Separation of butyl and pentyl benzoate without pulse damper (light blue), low volume (green) and high volume pulse damper (dark blue)

MATERIALS AND METHODS

The AZURA® HPLC plus system consisted of a low pressure gradient AZURA P 6.1L pump and an AZURA DAD 6.1L detector with a pressure proof flow cell. The column was replaced by a restriction capillary (ID 0.18 mm, length 15 m, back pressure ~ 90 bar).

For performance verification, a high pressure gradient AZURA P6.1L pump, and Autosampler AS 6.1L with a 20 µl sample loop and an AZURA MWD 2.1L with a 10 mm flow cell was used. The sample containing butyl and pentyl benzoate was separated on a Vertex Plus Column 150 x 4 mm Eurospher II 100-5 C18 with precolumn.

Tab. 2 Method parameters

	Damping efficiency	Gradient	Performance verification
Flowrate	0.5 mL/min 1 mL/min	1 mL/min	1 mL/min
Eluents	Water	A: Water B: Water with caffeine 0.015 g/L	A: Water B: Acetonitrile
Detection Wavelength	254 nm	272 nm	254 nm
Data Rate	10 Hz	10 Hz	10 Hz
Time constant	0.1 s	0.1 s	0.1 s

Tab. 3 Pump program (gradient)

Time [min]	% A	% B
0:00	100	0
2:00	100	0
2:06	90	10
5:00	90	10
5:06	50	50
8:00	50	50
8:06	10	90
11:00	10	90
11:06	0	100
14:00	0	100
14:06	100	0
18:00	100	0

Tab. 4 Pump program (performance verification)

Time [min]	% A	% B
0:00	25	75
0:50	25	75
5:00	5	95
6:00	5	95
7:00	25	75
10:00	25	75

CONCLUSION

The KNAUER pulse dampers are effective in dampening the residual pulsation from the system pump to up to 95 %. This results in very low pressure fluctuation and baseline noise. The gradient test results show that the KNAUER pulse damper has a very efficient mixing behavior. This is highly beneficial and timesaving during change of the mobile phase and while using a gradient method. With a signal to noise ratio improvement of up to 50 %, detection limits can be significantly lowered, which makes the addition of a pulse damper very attractive for the analysis of trace components.

REFERENCES

[1] John Dolan, Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer?, Waters Corporation, USA, 2018; http://www.waters.com/webassets/cms/library/docs/720005723en.pdf