Science with Passion

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Benefits of KNAUER´s trouble-free analytical capillaries in terms of HPLC and UHPLC system performance

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SUMMARY

KNAUER has introduced new capillaries, fittings and Start-Up Kits for all AZURA® Analytical HPLC and UHPLC/ULDC systems. The goal was to provide a "trouble-free" solution that is easy to handle and reusable many times. But does it affect the performance of the system?

INTRODUCTION

Every (U)HPLC user needs to install and replace capillaries and fittings on a regular basis. These comparably small and inexpensive supplies can have a major influence on both the user experience and the system performance. Both factors are important regarding the outcome of every single analysis.
KNAUER decided to use the well-established IDEX MarvelXACT™ capillaries and fittings for all AZURA® Analytical systems. They are finger tight up to UHPLC pressure, so that no tools are required. They auto adjust to various port depths and do not depend on ferrules. The sealing at the bottom of the port is achieved without complex techniques, which significantly reduces required torque **(Fig.1)**.

The trouble-free connection system eliminates the risk of under- and over-tightening, with a patented torque-limiting mechanism. This unique feature provides a haptic "click" feedback when it reaches the optimum torque, assuring a perfect connection for every user, independent from the expertise level. These capillaries can be connected and disconnected more than 100 times.

Fig. 1 Trouble-free connection system, sealing at the front side

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Start-up kits are available for any standard AZURA® Analytical system. These kits have been designed for most convenient installation with a comparable back pressure rating to the former used kits, providing equal or better system performance at the same time. The benefits of user experience are clear, but what about system performance compared to the conventional capillary/ferrule connection systems?
In this tech note, we show the results and unravel the mystery of whether this design worked as planned in laboratory reality.

SYSTEM BUILD-UP

Conventional K-Connect, which uses the classical system of ferrule and fitting, and trouble-free MarvelXACT™ connections were tested on an AZURA® Analytical HPLC 862 bar system and a UHPLC 1240 bar system. The system build-up **(Fig.2)**, HPLC method, solvents and sample were kept the same, only the capillaries and fittings were exchanged according to the instructions of the start-up kits. A well-established method used for system performance verification was used and adapted for these tests. After an isocratic run to determine the system performance based on the changed extra-column volume (ECV), also a gradient run was performed to see the impact of the changed dwell volume (see **Tab.1** and **Tab.2)**. The extra column volume is defined as the volume between the point of the injection and the point of detection. It relates to the band spread, and every connection, tubing, or fitting in the post-injection flow path contributes to the ECV. The dwell volume, also known as gradient delay volume, is determined by the pump characteristics. It is defined as the volume from the point of mobile phase mixing to the inlet of the column.

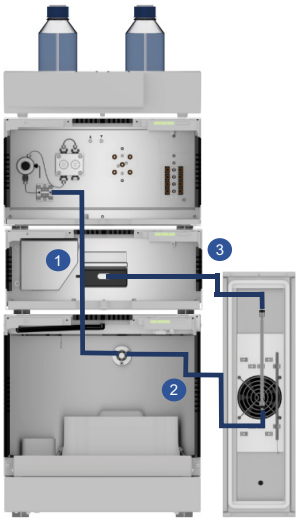

Fig. 2 AZURA® Analytical system build-up with installed capillaries 1, 2 and 3

Tab. 1 Capillaries for AZURA® Analytical HPLC 862 bar systems

		Former K-Connect system		New trouble-free system	
		Length [mm]	ID [mm]	Length [mm]	ID [mm]
1	Pump - Autosampler	700	0.18	600	0.254
2	Autosampler – HPLC Column	700	0.18	600	0.125
3	HPLC Column - Detector	400	0.18	500	0.125
	Resulting capillary impact on extra column volume	28.6 µl		13.5 µl	
	Resulting capillary impact on dwell volume	35.6 µl		37.8 µl	

Tab. 2 Capillaries for AZURA® Analytical UHPLC 1240 bar systems

		Former K-Connect system		New trouble-free system	
		Length [mm]	ID [mm]	Length [mm]	ID [mm]
1	Pump - Autosampler	700	0.1	600	0.254
2	Autosampler – UHPLC Column	700	0.1	500	0.075
3	UHPLC Column - Detector	400	0.1	350	0.075
	Resulting capillary impact on extra column volume	8.6 µl		3.8 µl	
	Resulting capillary impact on dwell volume	11.0 µl		32.6 µl	

RESULTS

For the AZURA® Analytical HPLC 862 bar system, the overall pressure including the column rose from 60 to 70 bar when changing to the trouble-free capillary system. The isocratic run to determine the system performance gave similar results for both capillary systems **(Fig.3)**. Retention times as well as theoretical plates and peak shapes were comparable **(Tab.3)**.

Tab. 3 Results for the AZURA® Analytical HPLC 862 bar system

	System pressure (including HPLC column)	Retention time Peak 1	Height Peak 1	Retention time Peak 2	Height Peak 2	Theoretical plates/m Peak 2
K-Connect	60 bar	5.18 min	41.07 mAU	7.14 min	27.89 mAU	84879
Marvel-XACT™	70 bar	5.04 min	42.57 mAU	6.92 min	28.73 mAU	84850

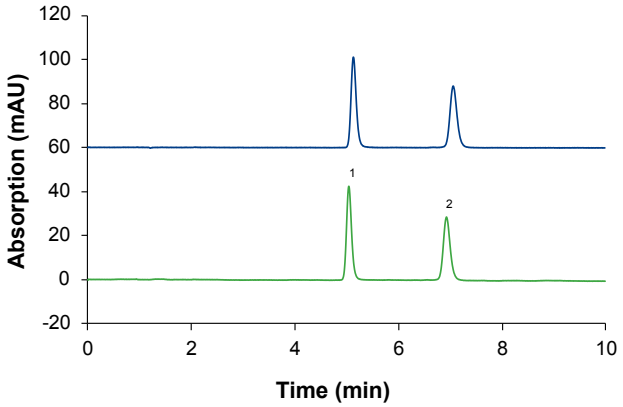

Fig. 3 Chromatograms of isocratic run on AZURA® Analytical HPLC 862 bar system; blue: K-Connect; green: MarvelXACT™; (1) Butyl benzoate; (2) Pentyl benzoate

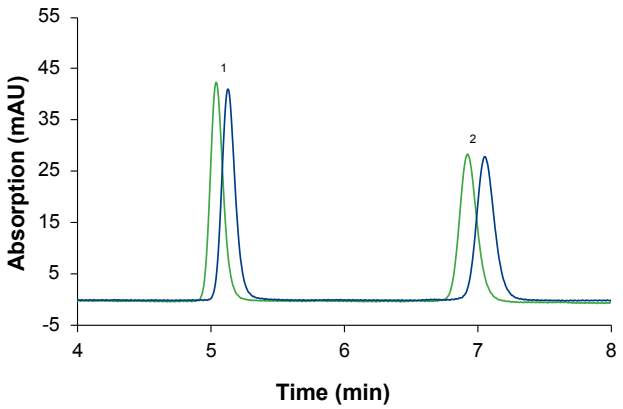

Fig. 4 Zoom in overlay chromatograms of isocratic run on AZURA® Analytical HPLC 862 bar system; blue: K-Connect; green: MarvelXACT™; (1) Butyl benzoate; (2) Pentyl benzoate

The small variation in retention time **(Fig.4)** can be explained by the lower extra column volume for the new capillary kits. As it is an isocratic run, the slightly higher dwell volume does not play a role here. The lower extra column volume should also result in a better performance and therewith a higher number of theoretical plates. The used HPLC column was chosen in standard HPLC dimensions of 150 x 4 mm with a resulting column void volume of around 1.28 ml. The extra column volume therewith only plays a minor role. The expected effect is more relevant in UHPLC where smaller columns are applied, and the extra column volume becomes more significant compared to the column void volume. As expected, there are no major differences in peak retention using gradient elution because the dwell volume does not differ significantly for both capillary kits **(Fig.5** and **Fig.6)**.

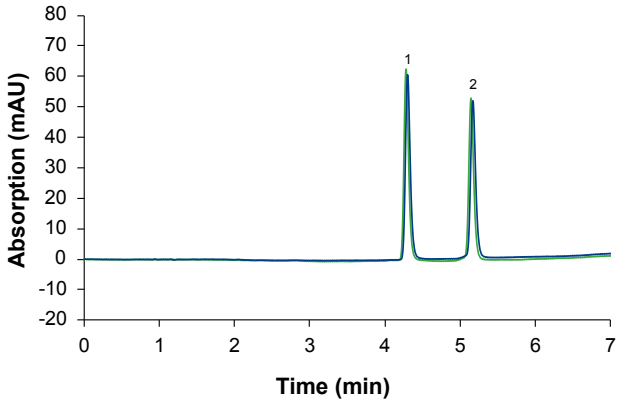

Fig. 5 Chromatograms of gradient run on AZURA® Analytical HPLC 862 bar system; blue: K-Connect; green: MarvelXACT™; (1) Butyl benzoate; (2) Pentyl benzoate

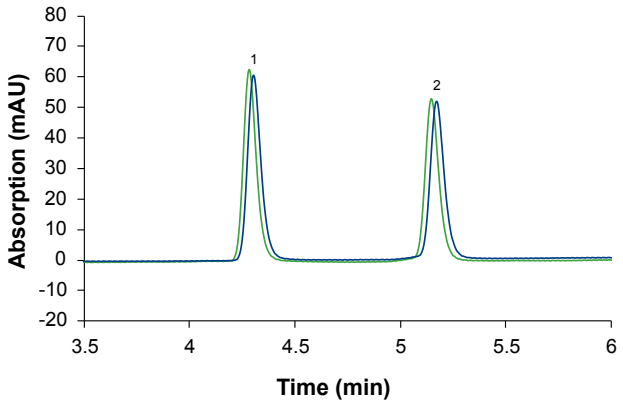

Fig. 6 Zoom in overlay chromatograms of gradient run on AZURA® Analytical HPLC 862 bar system; blue: K-Connect; green: MarvelXACT™; (1) Butyl benzoate; (2) Pentyl benzoate

Benefits of KNAUER´s trouble-free analytical capillaries in terms of HPLC and UHPLC system performance

For the AZURA® Analytical UHPLC 1240 bar system, the overall pressure including the column did not increase when changing the capillaries. The system performance was enhanced using the new capillary system. Retention times and peak shapes were comparable, while the number of theoretical plates was improved significantly with the new capillary system **(Fig.7** and **Fig.8)**.

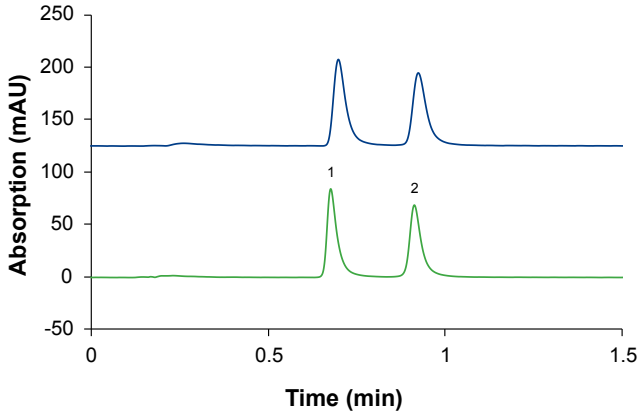

Fig. 7 Chromatograms of isocratic run on AZURA® Analytical UHPLC 1240 bar system; blue: K-Connect; green: MarvelXACT™; (1) Butyl benzoate; (2) Pentyl benzoate

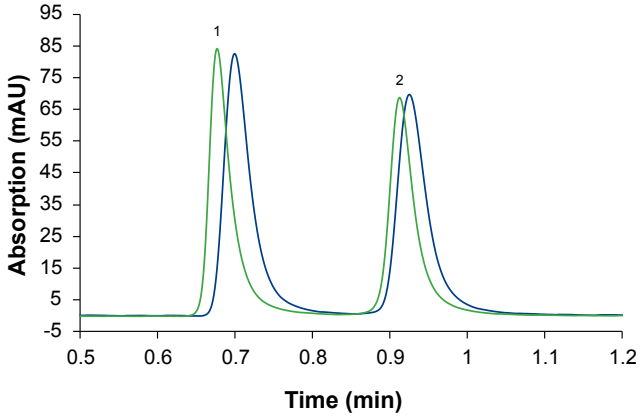

Fig. 8 Zoom in Chromatograms of isocratic run on AZURA® Analytical UHPLC 1240 bar system; blue: K-Connect; green: MarvelXACT™; (1) Butyl benzoate; (2) Pentyl benzoate

In UHPLC, the extra column volume plays a major role as column void volume becomes smaller. A common UHPLC column in the dimension 50 x 2 mm ID was chosen with a column void volume of about 0.11 ml. By reducing the extra column volume and especially by cutting the volume between column outlet and detector in half, a significant rise in theoretical plates was observed (see **Tab. 4)**.

Tab. 4 Results for the AZURA® Analytical UHPLC 1240 bar system

	System pressure (including HPLC column)	**Retention time Peak 1**	**Width W05 Peak 1**	**Retention time Peak 2**	**Width W05 Peak 2**	**Theoretical plates/m Peak 2**
K-Connect	380 bar	0.70 min	0.037 min	0.93 min	0.041 min	56858
Marvel-XACT™	380 bar	0.68 min	0.029 min	0.91 min	0.033 min	83032

For the gradient run, the results were similar for retention times of the two peaks **(Fig.9** and **Fig.10)**. This was expected, because the dwell volume does not differ significantly for both capillary kits.

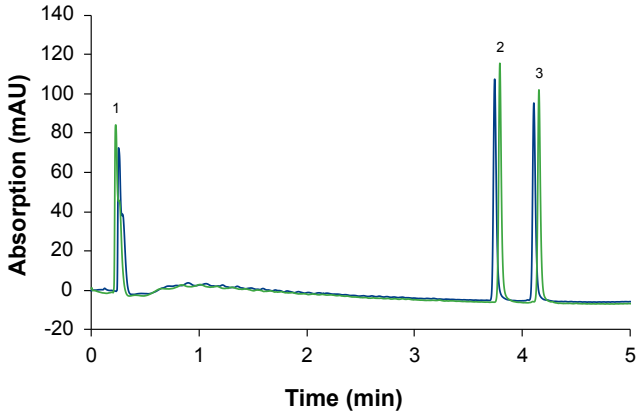

Fig. 9 Chromatograms of gradient run on AZURA® Analytical UHPLC 1240 bar system; blue: K-Connect; green: MarvelXACT™; (1) Injection peak; (2) Butyl benzoate; (3) Pentyl benzoate

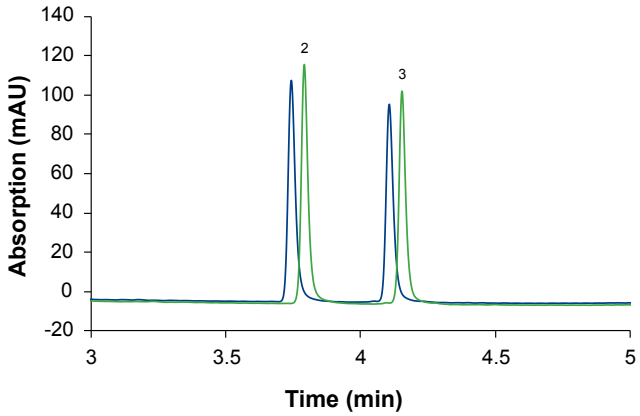

Fig. 10 Zoom in Chromatograms of gradient run on AZURA® Analytical UHPLC 1240 bar system; blue: K-Connect; green: MarvelXACT™; (2) Butyl benzoate; (3) Pentyl benzoate

In the gradient run, a little shift of retention times can be seen. With the new capillary system, both peaks elute a little later than with the former K-Connect system. In **Fig. 9**, also an injection peak can be seen. The injection peak is detected a bit earlier with the new capillaries compared to the former system. **Tab. 2** gives the explanation for these phenomena: The retention time of the injection peak is not affected by the eluent composition but as the extra column volume is smaller with the new capillary kit, the injection peak is detected earlier compared to the K-connect capillary kit. At the same time, the analytes are affected by eluent composition. This is one of the reasons why they can be separated on this stationary phase. Because the dwell volume is slightly higher with the new capillary kit, the higher eluting eluent composition arrives at the column later compared to the former capillary kit. If a smaller dwell volume and therewith a faster gradient formation is needed, capillaries with smaller volumes can also be applied and must be added to the standard start-up kit. Always be aware that they will have a smaller inner diameter which will result in an increase in the overall system backpressure.

CONCLUSION

Applying KNAUER´s trouble-free start-up kits for AZURA® Analytical HPLC and UHPLC/ULDC systems brings benefits to everyday laboratory life. The ease of use and the reusability of capillaries and fittings as well as the resulting system performance contribute to a positive user experience. These kits can replace the former recommended K-Connect kits without any limitations. Work tool-free and trouble-free while keeping the known HPLC or UHPLC system performance or even enhancing it.

MATERIAL AND METHODS

Tab. 5 System configuration AZURA® Analytical HPLC 862 bar

Instrument	Description	Article No.
Pump	AZURA P 6.1 L HPG, 10 ml SST pump head	APH35EA
Autosampler	AZURA AS 6.1L, Standard, 862 bar	AAA50AA
Column Thermostat	AZURA CT 2.1	ATC00
Column	Eurospher II 100-5 C18, 150 x 4 mm ID with integrated precolumn	15WE181 E2J
Detector	AZURA DAD 2.1L	ADC01
Flow cell	Analytical PressureProof Flow cell cartridge, 10 mm path length, 10 µl volume	AMC38
Capillaries K-Connect	Start up Kit including: Pump – Autosampler: Length 700 mm, ID 0.18 mm Autosampler – Column: Length 700 mm, ID 0.18 mm Column – Detector: Length 400 mm, ID 0.18 mm	- - - -
Capillaries MarvelXACT™	Start up Kit including: Pump – Autosampler: Length 600 mm, ID 0.254 mm Autosampler – Column: Length 600 mm, ID 0.125 mm Column – Detector: Length 500 mm, sID 0.125 mm	AZF120 AZF125 AZF115 AZF114
Software	ClarityChrom 9.0.0 – Workstation, autosampler control included	A1670
Software	ClarityChrom 9.0.0 – PDA extension	A1676

Tab. 6 System configuration AZURA® Analytical UHPLC 1240 bar

Instrument	Description	Article No.
Pump	AZURA P 8.1L, HPG, 5 ml SST pump head	APF45PA
Autosampler	AZURA AS 6.1L, standard, 1240 bar	AAA10AA
Column Thermostat	AZURA CT 2.1	ATC00
Column	Zorbax RRHD 1,8 µm SB-C18 50 x 2 mm	-
Detector	AZURA DAD 6.1L	ADC11
Flow cell	Analytical PressureProof Flow cell cartridge, 10 mm path length, 10 µl volume	AMC38
Capillaries K-Connect	Start up Kit including: Pump – Autosampler: Length 700 mm, ID 0.1 mm Autosampler – Column: Length 700 mm, ID 0.1 mm Column – Detector: Length 400 mm, ID 0.1 mm	- - - -
Capillaries MarvelXACT™	Start up Kit including: Pump – Autosampler: Length 600 mm, ID 0.254 mm Autosampler – Column: Length 500 mm, ID 0.075 mm Column – Detector: Length 350 mm, ID 0.075 mm	AZF110 AZF125 AZF05 AZF05-1
Software	ClarityChrom 9.0.0 – Workstation, autosampler control included	A1670
Software	ClarityChrom 9.0.0 – PDA extension	A1676

Benefits of KNAUER´s trouble-free analytical capillaries in terms of HPLC and UHPLC system performance

Tab. 7 Isocratic method for AZURA® Analytical HPLC 862 bar

Parameter	Description
Eluent A	Water
Eluent B	Acetonitrile
Gradient	Isocratic: Water/Acetonitrile 25:75 (v/v)
Flow rate	1 ml/min
Temperature	25 °C
Detection	UV 254 nm
Data rate	10 Hz
Time constant	0.1 sec
Injection mode	Partial loop
Injection volume	10 µl 0.1 mg/ml Butyl-/Pentyl benzoate

Tab. 8 Gradient method for AZURA® Analytical HPLC 862 bar

Time [min]	% B
0.0	75
0.5	75
5.0	95
6.0	95
7.0	75
10.0	75

Tab. 9 Isocratic method for AZURA® Analytical UHPLC 1240 bar

Parameter	Description
Eluent A	Water
Eluent B	Acetonitrile
Gradient	Isocratic: Water/acetonitrile 30:70 (v/v)
Flow rate	0.6 ml/min
Temperature	40 °C
Detection	UV 254 nm
Data rate	20 Hz
Time constant	0.05 sec
Injection mode	Partial loop
Injection volume	5 µl 0.1 mg/ml Butyl-/Pentyl benzoate

Tab. 10 Gradient method for AZURA® Analytical UHPLC 1240 bar

Time [min]	% B
0.0	10
5.0	90
5.1	10
7.0	10