

Introducing the KNAUER HTQC system solution for High Throughput Quality Control

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SUMMARY

High throughput usually means analyzing dozens, hundreds, or even thousands of samples per day in the laboratory or on a particular instrument. But how can this be achieved and what is necessary to perform fast, reliable and automated measurements? In this technical note we take a closer look at those requirements and how they are implemented in our **H**igh **T**hroughput **Q**uality **C**ontrol system, short HTQC.

INTRODUCTION

What does high throughput mean? And what parameters and hardware requirements are crucial for performing high throughput analyses? The term 'high throughput' in this context refers to the utilisation of automated technologies and processes to expeditiously execute a substantial number of tests or experiments in parallel. The primary functionality of the system is to rapidly generate substantial quantities of data.

Therefore, the key words for describing an HTQC system are speed, sample throughput, automation and miniaturization, as well as parallel processing. But how can this be achieved and what is necessary to perform fast, reliable and automated measurements? In this technical note we take a closer look at those requirements and how they are implemented in the KNAUER HTQC system.

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RESULTS

Speed

High throughput HPLC applications most often require fast methods. This could refer to both the overall method runtime and the cycle time of the injection module. Fast gradient applications up to 1200 bar can easily be realized with the HTQC system due to its two included UHPLC high pressure gradient pumps (Fig.1).

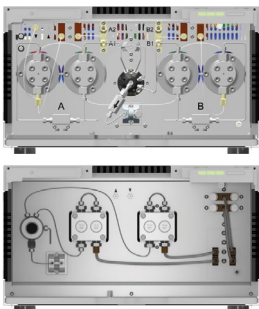
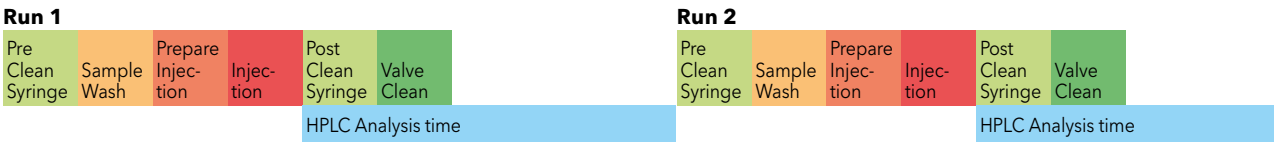


Fig. 1 HPG pumps of HTQC system, top: P 8.1L (max. pressure 1240 bar), bottom: P 6.1L (max. pressure 1000 bar)

Switching from classical HPLC to fast, high-resolution methods can significantly enhance your daily sample throughput. Assuming we are running an “old fashioned” HPLC method on a 250 x 4.6 mm ID column, the total runtime per sample is 75 min, including equilibration, analysis, and cleaning. This would allow for around six samples per day during an eight-hour working day.

Without Overlapped Injections



With Overlapped Injections

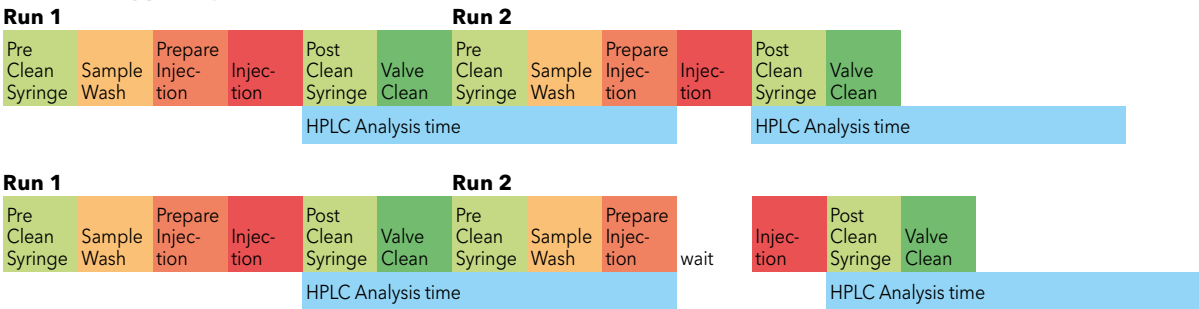


Fig. 3 Schematic buildup of injections with and without overlapped injections

Transferring the same method to UHPLC will be a significant productivity boost. With analysis time, equilibration time and backflushing time all reduced to less than six minutes per sample, now around 90 samples can be analyzed in a single working day. Another way of improving the speed of your analysis is to optimize your injection system. The cycle time of the injector can sometimes be very time-consuming due to washing steps and the injection procedure itself. The HTQC system is equipped with the KNAUER LH 8.1 Analytical Liquid Handler as standard (Fig.2).



Fig. 2 KNAUER liquid handler LH 8.1 (887 mm long rail version)

The LH 8.1 features the use of overlapped injections. That means you can effectively save time because the injection for the next run will be already prepared while the measurement is still in progress, without compromising the necessary cleaning steps of the injection system (Fig. 3).

Using the overlapped injection feature results in a saving of one more minute per sample. Together with the already implemented UHPLC method we can now analyze approximately 105 samples per day.

Sample throughput

Another crucial point for high throughput analyses is the number of samples your system can handle or store. Again, the LH 8.1 shows its benefits due to the opportunity to use up to three robotic coolers (**Fig. 4**) when the version with a longer rail is chosen. The use of three coolers with three drawers in each cooler allows you to store 1170 vials when you use 10x13 sample trays. Combined with the typical low eluent use of UHPLC, this setup gives us the possibility to extend our working hours from 8 to 24. The same UHPLC method, including the function for overlapping injections, can now be used around the clock. This allows around 320 samples to be processed per day.

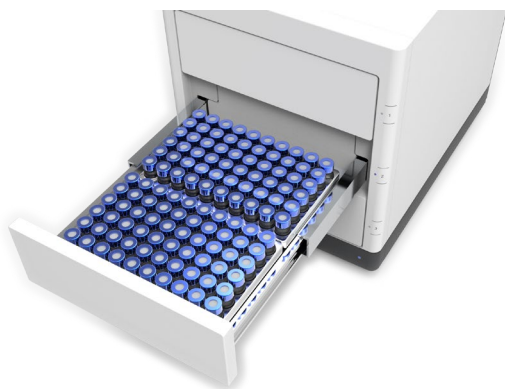
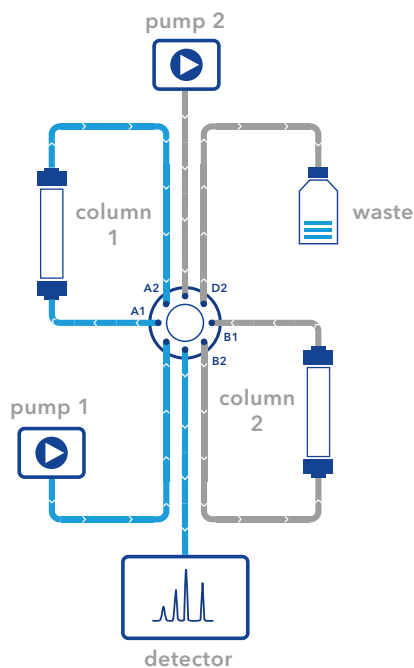


Fig. 4 Exemplary robotic cooler with two 10x6 trays

Automation

Besides the common 6 port-2 position injection valve, our HTQC system is equipped with an additional valve. This special column switching and backflushing valve allows you to operate two alternating columns in a single UHPLC system (**Fig. 5**). While one column is running the analysis, at the same time the second column can be backflushed and equilibrated in reverse flow direction. This is a major advantage for reducing matrix effects due to complex sample matrices typically found also in QC labs. Furthermore, this contributes to the overall runtime of the method.

Position 1



Position 2

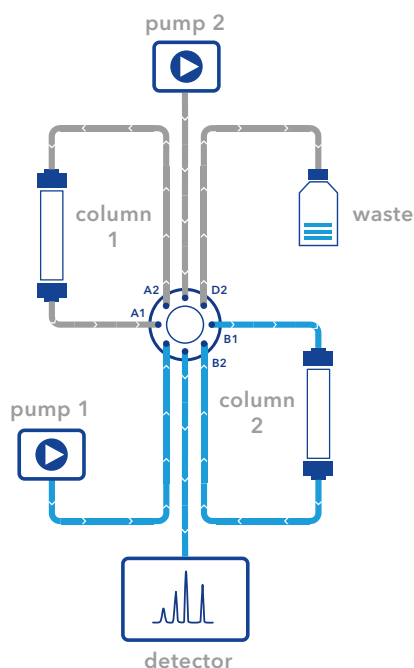


Fig. 5 Flow path for special backflush valve, top: Valve position 1: Column 1 in analytical stream, column 2 is backflushed; bottom: Valve position 2: Column 1 is backflushed, column 2 in analytical stream

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Parallel processing

In the last paragraph the special valve for backflushing and column cleaning was introduced. But to efficiently make use of its benefits, it is necessary to implement a second gradient pump to the system. The HTQC system solves this with an AZURA P 6.1L. Thanks to all the features and automation, now it is possible to clean one column while the other runs a sample (Fig. 6). Combined with all the optimisations, around 600 samples can be analysed per day.

CONCLUSION

What have we done to increase the throughput? First, a scale down from a classical HPLC to a fast UHPLC method was performed. Then, a second pump and a special column switching and backflushing valve were added to our UHPLC system. Combined with the liquid handler LH 8.1, its option for overlapped injections and its outstanding sample storing capabilities, the HTQC system is a versatile configuration for high throughput analysis. It can be coupled to almost any detector that can be used in HPLC. Due to the possibility of very fast operation, mass spectrometric methods are a perfect fit!

	Run 1					Run 2				
LH 8.1state	Pre Clean Syringe	Prepare Injection	Injection	Post Clean Syringe	Valve Clean	Pre Clean Syringe	Prepare Injection	Injection	Post Clean Syringe	Valve Clean
Column1	Equilibration			HPLC Analysis		Cleaning				
Column2	Cleaning					Equilibration			HPLC Analysis	

Fig. 6 Schematic illustration of column cleaning, equilibration and analysis with overlapped injections and special backflush valve

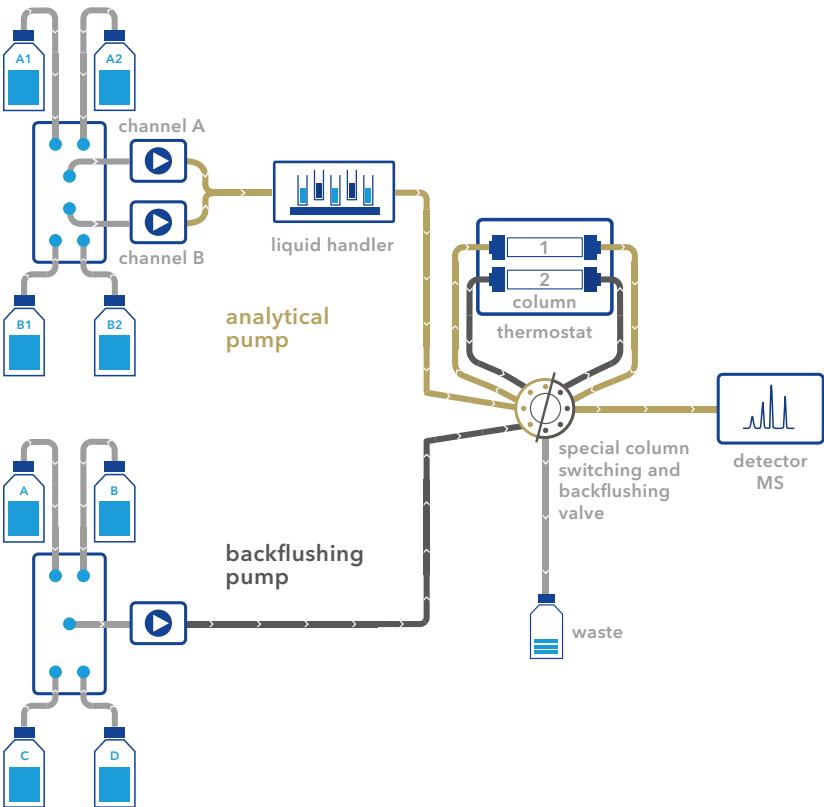


Fig. 7 Complete flow scheme of HTQC system

MATERIAL AND METHODS

Instrument	Description	Article No.
System	KNAUER AZURA HTQC UHPLC 1240 bar system for High-throughput quality control	499 005 205
	<ul style="list-style-type: none">• AZURA P 6.1L• AZURA P 8.1L• Liquid Handler LH 8.1• Column thermostat CT 2.1• Valve Unifier• Column cleaning and backflushing valve	