

# Automation in preparative chromatography - discover the device best suited for your application

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## SUMMARY

In many cases, automation of preparative purification is necessary to increase throughput. This can be easily achieved by integrating an autosampler and fraction collector or by using a liquid handler. The question then becomes which instrument is best suited to your application. Here we have provided a comparison of the liquid handler with the preparative autosampler and fraction collector to help users identify the most appropriate system configuration for their purification process.

## INTRODUCTION

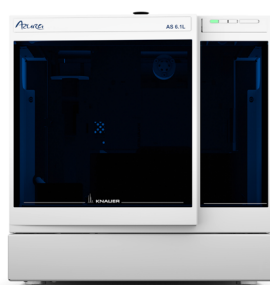
The goal of preparative-scale chromatography is the production of a quantity of compounds at a required purity and/or yield with the highest throughput. Preparative chromatography is widely used within the pharmaceutical and chemical industry to produce medicinal products, pharmaceutical ingredients, food additives or supplements to name a few. Throughput is also one of the most important parameters in preparative chromatography. The ability to automatically purify multiple samples saves time in large-scale purification workflows. It also increases efficiency by reducing manual intervention and allows continuous operation, increasing the throughput of the purification process. Automation minimises human error and ensures reproducible sample injections, leading to more consistent results. Sample application is a critical step in the purification process because of the need to inject large volumes of sample and/or multiple different samples to increase throughput. Numerous options for sample application are possible and will be shortly discussed. The simplest

and most attractively priced choice is an injection valve that is controlled either manually or operated by a valve drive. For single purifications with small sample volumes this is a very common solution. Nevertheless, during preparative separations large sample volumes might be the limiting factor for the use of an injection valve. Common alternatives for the application of large sample volumes are via a sample pump or by the system pump by using one designated channel of the system pump. However, injection via a valve or the pump is also limited when automating the process to purify multiple samples without manual handling. KNAUER offers two versatile options for automated sample injection: a classical preparative autosampler and a liquid handler that can also be used for fractionation. For a better understanding and classification of the instruments, we have prepared a table comparing the most important differences and advantages. We will explain the main functionalities of the instruments to help you decide which instrument is best suited for your application.

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Tab. 1 Comparison of the preparative autosampler and liquid handler

## Preparative Autosampler AS 6.1L



## Liquid Handler LH 2.1



<b>Focus</b>	Entry level automation option	Automating preparative separations with highest flexibility and throughput
<b>Best used for</b>	Small-scale and/or semi-preparative purifications	Preparative separations and small-scale production
<b>Purification range</b>	Milligrams	From milligrams to several grams
<b>Typical application</b>	Protein purification	Peptide and oligonucleotide purifications
<b>Benefits</b>	<ul style="list-style-type: none"> <li>• Biocompatible available</li> <li>• Cooling option</li> <li>• Pre-configured for ease of use</li> <li>• Small footprint</li> <li>• Can be integrated in the AZURA system tower</li> </ul>	<ul style="list-style-type: none"> <li>• Combines injection and fraction collection</li> <li>• High throughput</li> <li>• High Flexibility</li> <li>• Use your own racks</li> <li>• Define injection parameters</li> <li>• Reinjection of fractions</li> </ul>
<b>Injection range</b>	Up to 10 ml	Up to 60 ml
<b>Injection mode</b>	Partial loop fill up to 100 % loop volume	Partial loop fill up to 100 % loop volume Sandwich injection
<b>Sample loss</b>	Minimum sample loss of 60 µL	No sample loss*
<b>Max. number of samples</b>	30 x 10 ml 108 x 1,5 ml 192 x 96-deep well micro titer plates	Exemplary 160 x 50 ml 490 x 15 ml 810 x 2 ml 1440 (15x 96-deep-well microtiter plates)
<b>Washing</b>	Low carry-over due to efficient needle wash	Washing parameters adjustable up to 4 wash solvents
<b>Fractionation</b>	Not included	Included
<b>Supported software</b>	ClarityChrom OpenLab Chromeleon PurityChrom 5/6 Mobile Control	PurityChrom 5 Others on request

\*depending on injection mode

**Injection with the autosampler in the preparative mode**

The KNAUER AS 6.1L preparative autosampler is a pulled loop autosampler. This means that the sample is introduced into the sample loop by aspirating the sample with a syringe (Fig. 1). During the actual injection process the sample loop switches into the pump flow path. In the full loop injection mode of the analytical autosamplers, the sample loop is overfilled with sample by at least 1.5 times the loop volume to ensure the highest precision. The preparative autosampler only supports partial loop filling mode to avoid unnecessary sample loss. In partial loop filling mode, the sample loop is filled with both sample and wash solution. It ensures the highest accuracy of sample volume with minimal sample loss. Unlike the analytical autosampler, the maximum injection volume of the partial loop injection mode is

not limited to 50 % of the loop volume. It is possible to fill the sample loop completely and inject the entire sample loop volume. The fixed flush volume of the preparative sample needle is 60 µL, corresponding to the needle volume and therefore the minimum sample loss during injection. The preparative autosampler is available with biocompatible wetted parts and the ability to cool samples making it the ideal instrument for the purification of temperature-sensitive proteins or other molecules. The small footprint allows integration in the AZURA® system tower. It is very easy to use thanks to the pre-configured instrument in a variety of supported software packages. Due to the maximum injection volume of 10 ml the preparative autosampler is best used for small-scale and/or semi-preparative purifications in the milligram range.

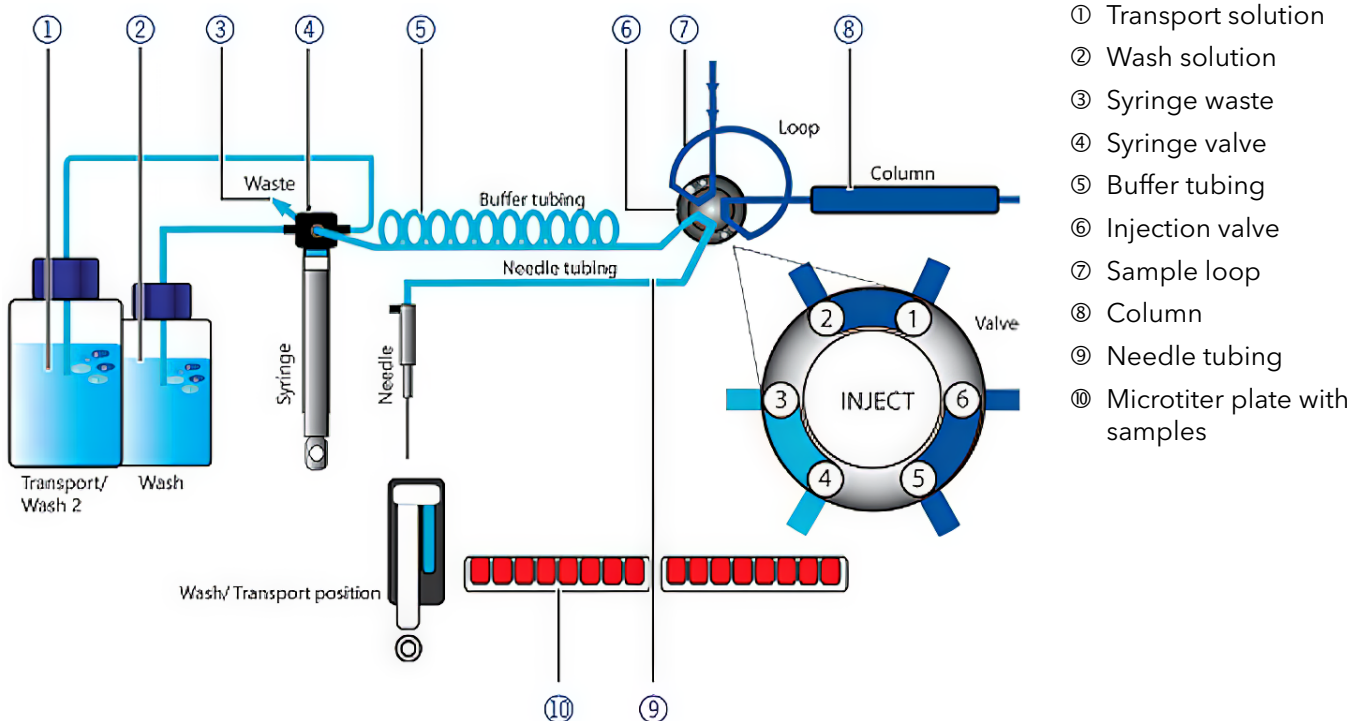


Fig. 1 Flow chart of preparative Autosampler AS 6.1L

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## Injection with the liquid handler

The liquid handler is as well a pulled loop injector that supports partial loop fill. The scheme of the injection path is shown in Fig. 2. The liquid handler is due to its larger injection volume and huge area for different racks ideal for automatized purifications up to the gram range and preparative applications where high throughput is required. Additionally sandwich injection is supported. Here the sample is located between two fluid segments

protecting your valuable sample from harsh wash solutions. Injection without sample loss is possible as the user can choose between air, sample, or sandwich solution to compensate for the dead volume of the needle tubing. The biggest difference is the combination of injection and fraction collection with freely configurable racks either for sample injection, compound collection and the possibility to reinject valuable fractions.

### Sample Injection

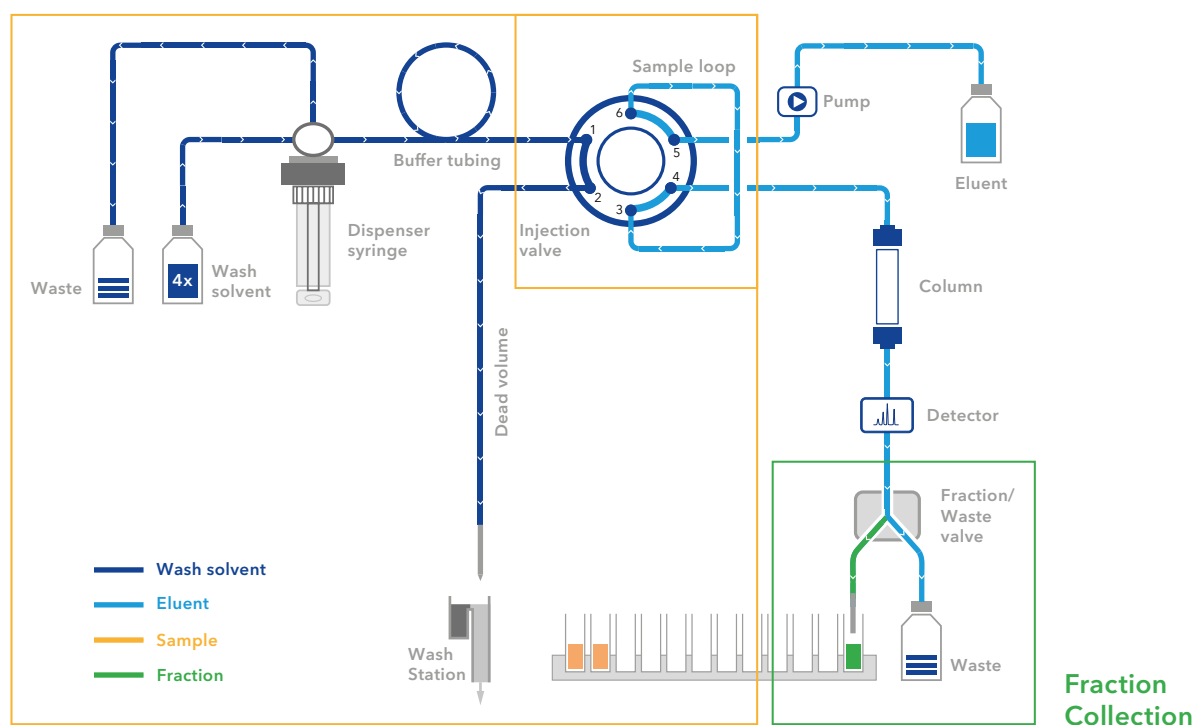



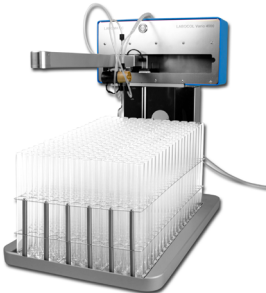

Fig. 2 Flow chart of Liquid Handler LH 2.1

## Fractionation

The aim of preparative separation is to purify a compound and collect fractions for further processing or analysis. During separation, the sample elutes from the column and is identified by a detector. Samples can be collected based on specific detection parameters. However, in preparative chromatography it is not uncommon to overload columns, resulting in poor separation and difficulty in collecting sharp peaks with a high purity

product. In many cases, large numbers of small fractions are collected and analysed to determine the sample composition with the desired purity and yield. Depending on the aim of the purification and expected amount of collected samples different fractionation options might be needed. We have prepared a table comparing the different fraction collectors and liquid handler to help you find the most suitable instrument for your application.

Tab.2 Comparison of fraction collectors

	FC 6.1	LABOCOL Vario 4000	Liquid Handler LH 2.1
			
<b>Focus</b>	Entry level fractionation option	Versatile fractionation for higher flow rates	Automating preparative separations with highest flexibility and throughput
<b>Best used for</b>	Small-scale and/or semi-preparative purifications	Preparative separations and small-scale production	Preparative separations and small-scale production
<b>Typical application</b>	Protein purification	Small molecule purification	Peptide and oligonucleotide purifications
<b>Benefits</b>	<ul style="list-style-type: none"> <li>• Biocompatible version</li> <li>• Cold room friendly</li> <li>• Fits on top of AZURA device</li> <li>• Height adjustable</li> <li>• Racks freely accessible</li> </ul>	<ul style="list-style-type: none"> <li>• High fraction capacity</li> <li>• Flexibility</li> <li>• Use your own racks</li> <li>• Enclosure available</li> </ul>	<ul style="list-style-type: none"> <li>• Combines injection and fraction collection.</li> <li>• Highest throughput and fraction capacity</li> <li>• Reinjection of collected fractions</li> <li>• Flexibility</li> <li>• Use your own racks</li> </ul>
<b>Maximum samples/ Exemplary for 15 ml tubes</b>	One Rack: 99 Tubes + side rack with 3 x 250 ml	Vario (three racks): 240 tubes Vario Plus (five racks) 400 tubes	Five racks: 490 tubes*

\*Depending on number of used racks for fractionation and injection

## CONCLUSION

When purifying a compound by preparative chromatography, the injection and collection of the sample, together with the separation on the column, are key to successful purification. The ability to automate this process is highly recommended as it reduces manual interaction and therefore costs and error. KNAUER offers a variety of options for injection and fractionation allowing automation and continuous operation, increasing the throughput of the purification process. Small-scale preparative automation can be achieved by combining

the preparative autosampler AS 6.1L with a small or medium capacity fraction collector such as FC 6.1 or LABOCOL Vario 4000. In addition, high throughput and high-capacity applications can be realised with the Liquid Handler LH 2.1, which combines autosampler and fraction collector functions.