

HPLC & UHPLC

Separate, purify, go!

Separation power paired with precise fractionation with the Vanquish Analytical Purification LC System

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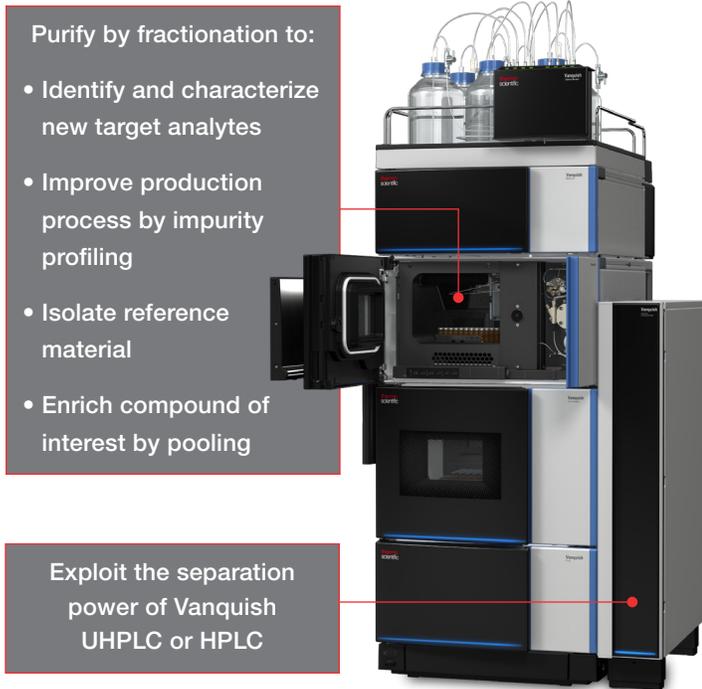
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Fraction Collection, HPLC, High Performance Liquid Chromatography, UHPLC, Method Development Systems, Vanquish Core, Vanquish Flex, Vanquish Horizon, Automated Delay Volume Determination, Vanquish Fraction Collector

Introduction

The isolation of a single compound from a complex mixture can be a major challenge for scientists tasked with in-depth sample characterization. The demands for isolation followed by further investigation can be multifaceted, based on the key area of focus of the drug development process. For example, in drug discovery, it can be used for the identification of a new active substance (e.g., a component in a traditional medicine), or assist with the screening of compound libraries for leads. For manufacturing, it can be used for improving the process of new drug synthesis by investigating the side products like in gene therapy, or for quality control, where it can be used for evaluating the purity of incoming goods or even final products. Overall, the purity of isolated products is key for the success of processes connected to the fractionation step.

Typically, analytes are first resolved by chromatographic techniques in the analytical flow range and samples are then obtained using a fraction collector. A major challenge is to ensure that the resolution of compounds resulting from the chromatographic separation is preserved in the fractionated samples—ideally, each fractionated sample will contain just one pure compound. The Thermo Scientific™ Vanquish™ Analytical Purification LC system readily meets this challenge, ensuring that the resolution of even an UHPLC separation is precisely coupled with rapid and accurate sample fractionation, in one powerful, flexible, easy to use platform (Figure 1).



The Vanquish Fraction Collector and the LC System

The heart of the Vanquish Analytical Purification LC system is the integrated Thermo Scientific™ Vanquish™ Fraction Collector. It is designed to tackle the most important aspect of fraction collection—to maintain the peak resolution of the chromatographic separation in the collected fractions. Failure to precisely collect fractions can result in cross contamination affecting both purity and concentration measurements, as well as the next steps of analysis.

The key to achieving the pinpoint isolation of compounds from high-performance separations is to know (and minimize) the exact time taken for an analyte to travel from the detector to the sample vial to precisely cut the fractions. With this information, maintaining the high resolution of the separation as well as achieving high recovery with minimizing loss of precious compounds, is possible.

Therefore, the Vanquish Analytical Purification LC system is specifically engineered to ensure optimal fraction collection with minimized delay volume by keeping capillary connections as short and internal volumes as small as possible. The system also takes into account the method conditions of the application while facilitating the appropriate fraction size and optimal fractionation trigger depending on the needs of the dedicated application (Figure 2).

Figure 1. The Vanquish Analytical Purification LC system features the integrated Vanquish Fraction Collector, pairing LC separation power with precise fractionation.

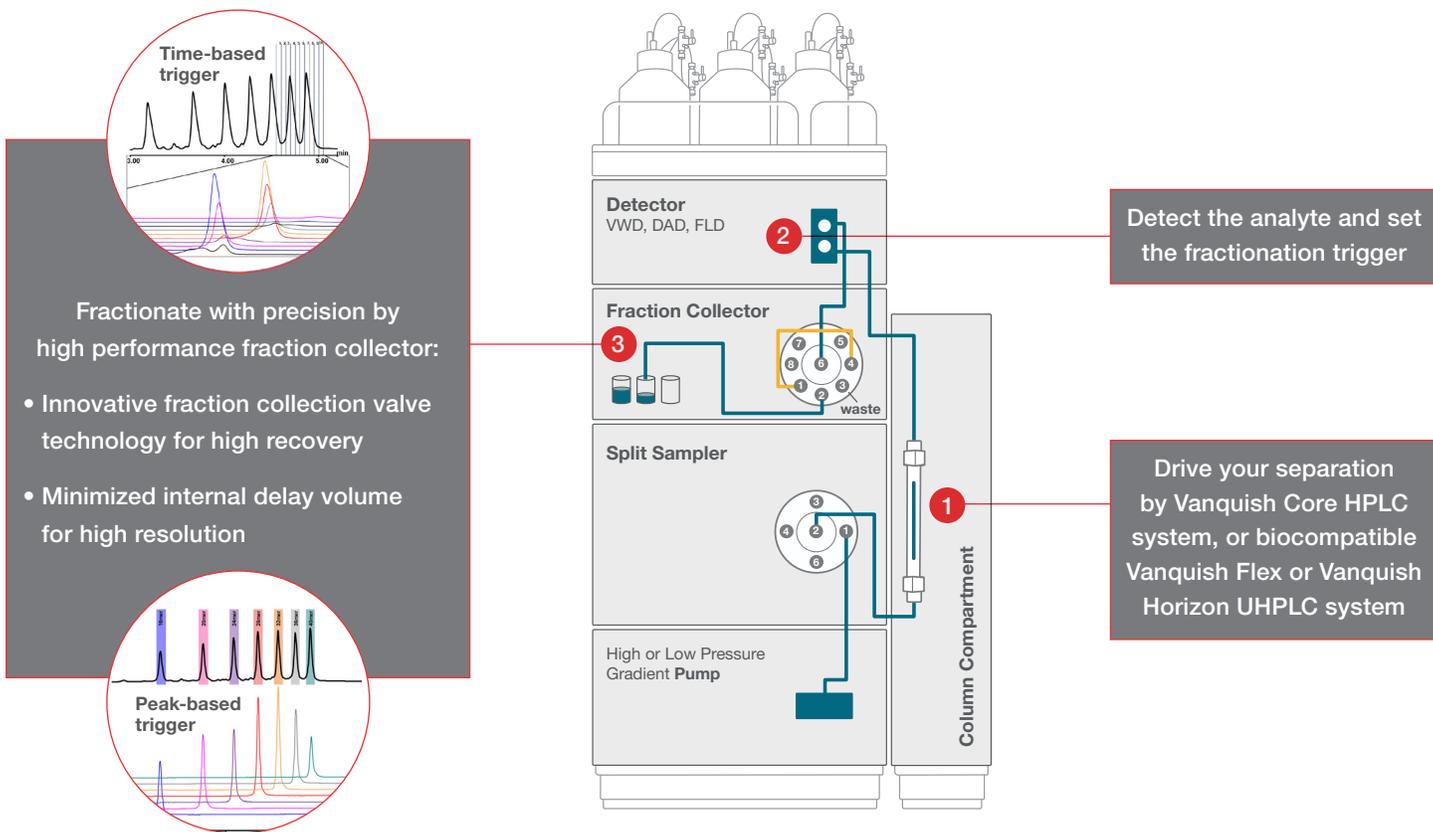


Figure 2. Fractionation with precision after high performance separation is achieved coupling the LC to an innovative fraction collection valve, guiding the compounds through optimized fluidics. The minimized internal delay volume after peak detection to the tip of the dispensing needle ensures high recovery and high resolution at the same time.

Defining the targeted fraction size and fractionation process is dependent on several factors and following questions need to be answered: How much of the sample or relative amount of analyte to be isolated is present? How much do I need for the next step and how much can be loaded on a column with a single injection while not disturbing the chromatography? What column size do I need for the purification?

The flexibility of the Vanquish Analytical Purification LC system is broad with the option to pair the Vanquish Fraction Collector with:

- Thermo Scientific™ Vanquish™ Core HPLC system for routine and reliable purification up to 10 mL/min
- Thermo Scientific™ Vanquish™ Flex UHPLC system for flow rates up to 8 mL/min and the need for biocompatibility
- Thermo Scientific™ Vanquish™ Horizon UHPLC system for low abundant analytes out of complex mixtures, which are isolated after refined low-volume peak separations at flow rates down to 50 µL/min

Accurate fractionation requires the precise triggering of the fraction collector, which can be based either on elution time or detector signal. A UV or fluorescence detector can be located between the column and the fraction collector. For compounds lacking a chromophore, either a charged aerosol detector or a mass spectrometer might be the detector of choice. Due to their destructive nature a portion of the flow from the column must be diverted to them using a T-connector.

The heart of purification success: the fraction collection valve

A critical component is the fraction collection valve that has a very low internal volume and fast switching capacity (Figure 3). It uses the tool-free Thermo Scientific™ Viper™ Fingertight Fitting systems with near zero dead volume connections. These reliable and reproducible LC connections used throughout the complete system contribute to the exceptional performance of the purification system. The fraction collection valve also facilitates an extensive choice of flush, rinse and wash functions to prevent possible carry over and minimize sample cross contamination to ensure the isolation of target analytes with high purity and recovery.¹

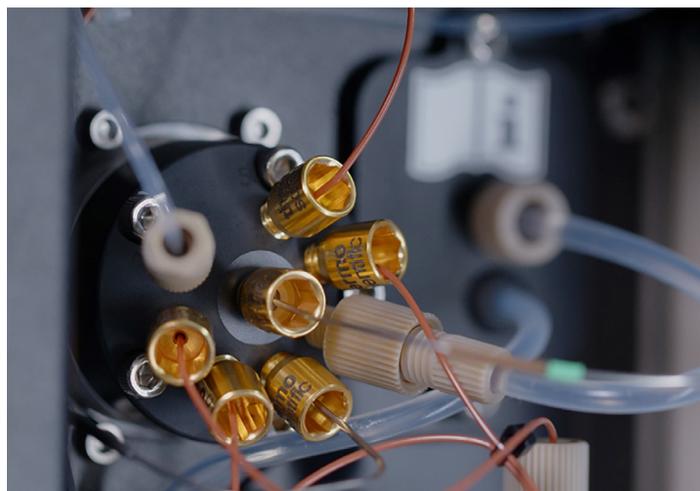


Figure 3. The heart of precise purification is the integrated Vanquish Fraction Collector with its low internal delay volume fraction collection valve and Viper Fingertight Fitting systems

Achieving full application flexibility by precisely engineered and optimized fluidic components

Besides the importance of the fraction collection valve, other factors play crucial roles for achieving superior purification performance. For example, the precise positioning of the needle and the speed and accuracy of needle movement provide high versatility to support a variety of custom collection methods to address their application needs. Typically, many fraction collector instruments position the dispenser tip to collect above the vessel. A continuous flow is coming out of the needle as it moves across the top of the containers. When using 96-well plates where the wells are next to each other this allows the entire collection time to be placed in the wells with no gaps. However, for compounds that are light sensitive, or if dust or evaporation is of concern, it is prudent to fractionate directly into a collection vessel with a closure, such as an amber vial sealed with a septum, or a well plate with a cover. Furthermore, collection directly into a container is essential when high flow rates are fractionated, and a stream is generated to prevent splashing of liquid. Cross contamination is thus avoided if the needle tip is lowered into the fraction collection vial, and a puncture offset is applied to avoid any pressurization of the collection vial. Another example for the use of fractionation directly into the vial uses the exact height setting of the dispensing needle to precisely control how eluent droplets fall, by allowing the fraction needle tip to touch the liquid level. The flexibility of needle positioning above or in vial guarantees the integrity of fraction collection (Figure 4).

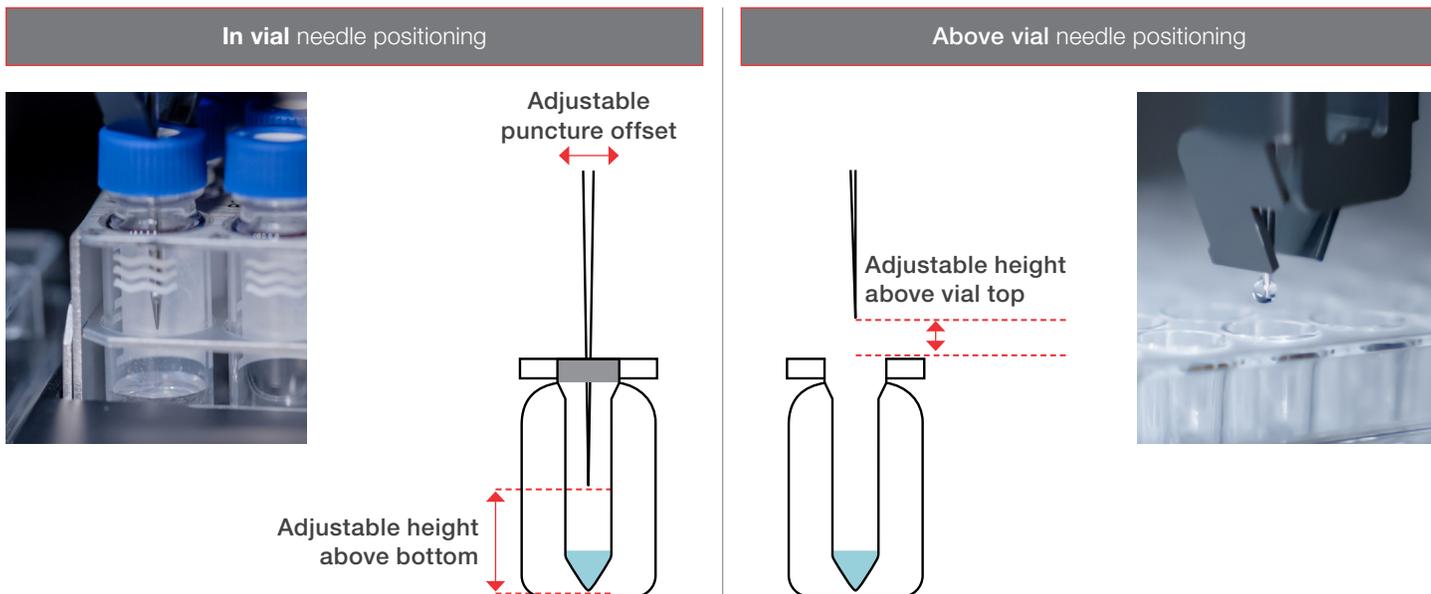


Figure 4: Precise needle positioning paired with speedy and flexible needle movement paths guarantee high purity, high recovery and minimized cross-contamination for a wide range of applications

In addition to precise needle positioning, the speed at which the needle moves to a new position is also important to minimize sample loss or cross contamination due to dripping. Needle movement from one position to the next, might be in a horizontal or row-like pattern, or a vertical or column-like pattern (Figure 5). By using the meander—or saw-shape path, the user has the full flexibility to control the fraction collection steps for optimized purification results depending on the application parameters chosen, which might encompass flow rates from low microliter to several milliliters per minute.

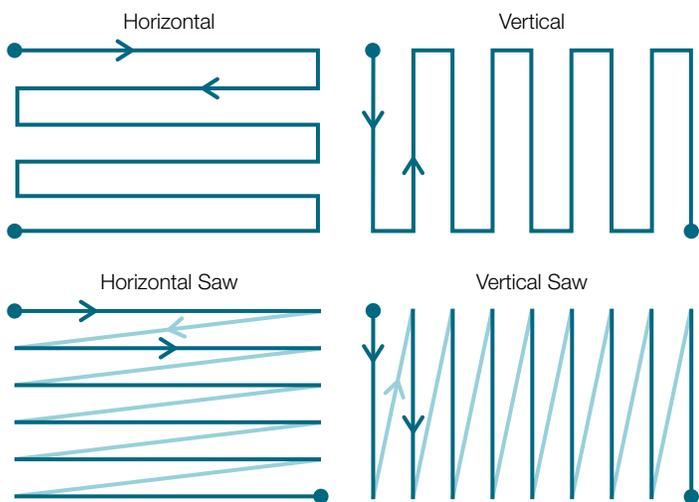


Figure 5: Flexibility in fractionation pattern for optimized fractionation steps tailored for the application demands

Your work matters: preserve your fraction integrity

With the Vanquish Analytical Purification LC system flexibility is key. Users can tailor every aspect of the purification process to meet their needs. This is reflected in the compatibility of the system with a wide variety of sample containers, which can be used both for injecting the sample and for collecting fractions. The user is not limited when choosing sample containers as the Vanquish Fraction Collector can handle a wide variety of individual vials, caps and well plates. This allows the easy transition from low volume collection containers to high volume collection containers in the same sample compartment. Subsequently, methods with different sized containers can be run on the same system to collect into the format required for the current method. Furthermore, the ability to use a variety of containers allows an easy transition of the collected fractions to the next step in the process, which might be scaling up or down from 384 well plate to 10 mL vials and back.

Regardless of what sample container format you choose, sample integrity is guaranteed in the Vanquish Analytical LC Purification System with proven features found in the Vanquish Autosamplers including integrated bar-code reading for fail safe recognition of the rack or well plate format, fraction compartment thermostating including heating or cooling, and the option to protect photo-sensitive isolated products against light. The contamination by dust is not a concern, as the fractions are not only protected by the closed compartment, but also with the ability to fractionate directly into the fraction collection vessels closed with septum or well plates with mats (Figure 6).

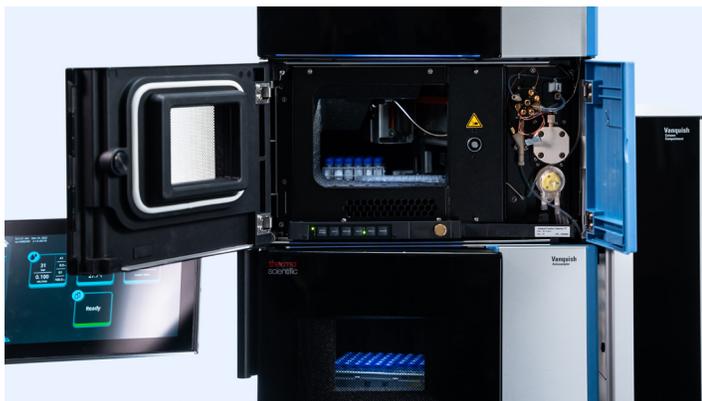


Figure 6. No matter which fraction vessel format you choose from our broad offering, the fractionated sample integrity is guaranteed by storing the containers in a temperature-controlled compartment, protected against light and contamination at the same time

**Focus on the next step:
simplified user experience by intuitive software tools**

The Vanquish Analytical Purification LC system is designed with ease of use in mind. As mentioned above, the information about the delay volume is critical. This can be calculated manually or, more conveniently measured, by using the automated Delay Volume Determination (DVD) function, which is included as an automated workflow with user guidance in the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software

(Figure 7). During the measurement, an air bubble is injected by the autosampler. The time taken for the air bubble to be detected first by an optical detector and then by an integrated air bubble sensor in the fraction collector module, is used to accurately determine the total delay volume. This simple and effective approach does not need any additional reagent like a dye and works easily with mobile phases available in every laboratory. The measurement leads to more precise results considering tolerances in the system, than just estimating based on theoretical calculations. The determined delay volume is applied in the Chromeleon Instrument Method automatically considering method specific settings contributing to exact switching time—the flow rate of the pump and the detector response time.

After knowing the exact delay volume, it is important to set the cutting of fractions by the fraction collector precisely in Chromeleon. How to choose the optimum settings for switching the valve and moving the needle to achieve a maximum purification of the analytes of interest might be a challenge at first glance. However, the intuitive step-by-step instrument method wizard helps novice and experienced users to set the fractionation steps appropriately, based on the chosen trigger for fractionation (using either time or detector signal). The operator can use the software's interactive fractionation simulation window to easily visualize the effects of their chosen parameters on fractionation performance (Figure 8).

Guided steps allow for exact delay volume determination

Based on the actual configuration

Startup	Name	Status	Start After	Queued By
▶	\\C:\Chromeleon\Local\Instru_VFC_DELAY_VOLUME	Running		stabel

Last Injected	Sequence Name	
3/24/2022 2:58:59 PM	01h_Flex_fast	Chromeleon\Local\Video\
3/24/2022 4:40:46 PM	01_Flex_long	Chromeleon\Local\Video\
	OQ_STOP	Chromeleon\Local\Instrum
	OQ_VFC_DELAY_VOLUME	Chromeleon\Local\Instrum

More Options: Fraction Collector VF-F20-A
Instrument: VQ_FlexPurification

General Settings

Needle Positioning Mode: AboveVal
 Height of 96 Well Plate: 50.0 [mm]
 Height of 384 Well Plate: 50.0 [mm]
 Puncture Offset: 0.0 [mm]

Above Vial Collection

Use Safe Needle Height: Enable
 Needle Height for Vials: 50.0 [mm]
 Needle Height for Well Plates: 50.0 [mm]

In Vial Collection

Needle Height for Vials: Automatic
 Needle Height for Well Plates: Automatic

Wash & Rinse

Wash Mode: NoWash
 Wash Time: 2.0 [s]
 Wash Speed: 24.0 [μl/s]
 Wash Pump: Off
 Rinse Mode: NoRinse

Delay Volume

Delay Volume: 7.9 [μl]
 Delay Capillary IDxL: 100umx350mm
 Delay Capillary ID: 100 [μm]
 Delay Capillary L: 350 [mm]

Pusher

Service... GetPusherStatus Close

Figure 7 : Automated delay volume determination to achieve highest precision in fraction collection by exactly timed fraction collection valve switching

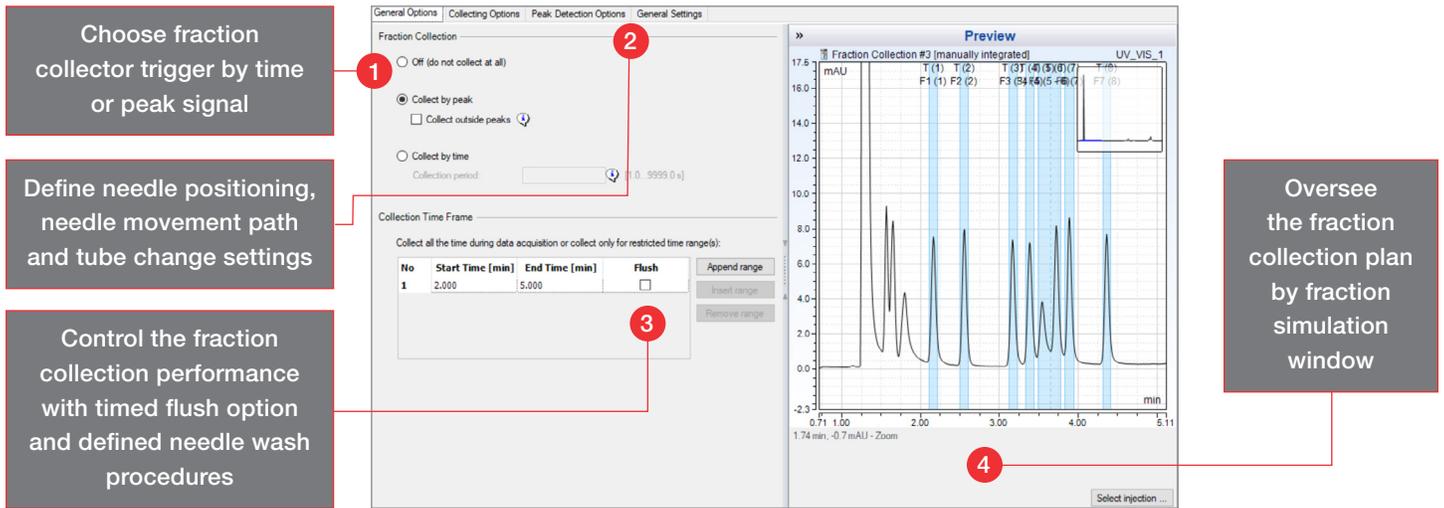


Figure 8. Intuitive step-by-step instrument wizard guides the novice and expert user through the optimized set-up of the fraction collection. High versatility enables the user to exactly tailor parameter options to meet their application needs.

After successful separation with fractionation, you are ready for the next step—data processing. This is facilitated by the intuitive user interface of the Chromeleon studio view. The positions of the fraction collection vials are conveniently displayed in the chromatogram, as well as visualized in the fraction tray view. This allows the user to easily connect the filled vials in the fraction

collector with the detected peaks. A report, which can be easily customized, contains details about the collected volume, the collected time frame and the detected peak. The user can use this information to prepare for the next step in their process such as quantitation or qualification of the collected reference material (Figure 9).

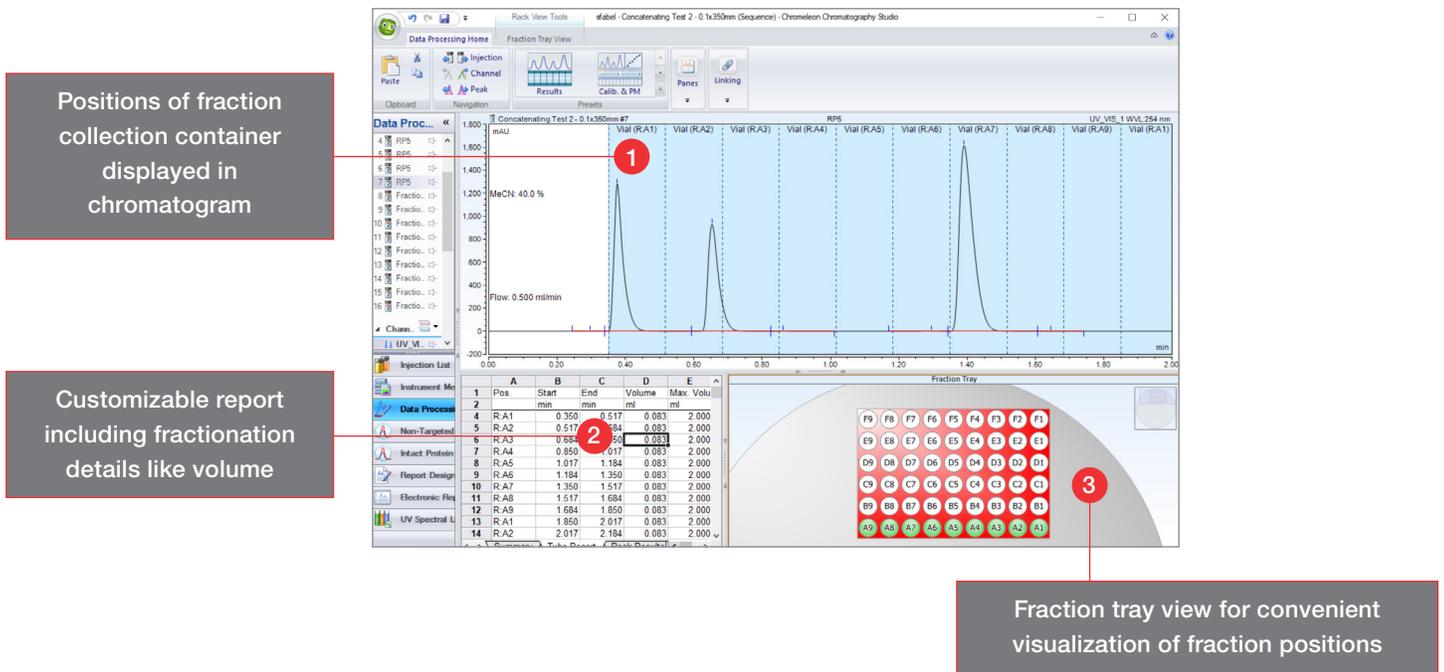


Figure 9. The Chromeleon Console view facilitates the visualization of the fraction collection result, with displaying the fractionation of the peaks in the chromatogram, displaying a report and the fraction tray view to allow you to focus on the next step of work that matters

Conclusion

Get the purest sample for you next step with pinpoint purification

Whether you need to gain new insights into your process, or if you are on the path to scientific progress, the Vanquish Analytical Purification LC Systems allows you to focus on the next steps for your continued success. The system seamlessly marries advanced chromatographic separation power with highly precise fractionation using integrated fraction collector, and provides wide application flexibility. By design the Vanquish Fraction Collector supports multiple modes of fractionation, uses precise needle positioning and needle movement, and includes a rapidly switching fraction collection valve to achieve pinpoint purification. The system is extremely flexible and can be used from low to high analytical flow rates, triggered by time or detector signal, and fractionated samples can be collected into a wide variety of sample containers. High recovery and minimal carry-over is achieved by intelligent fluidic flow path design with flush, rinse and wash option, as well as smart features like automated delay volume determination and intuitive instrument method wizards. The Vanquish Analytical Purification LC System enables you to easily separate, purify and run your process (Figure 10).

References

1. Thermo Scientific Technical Note 72940 , Principles of fraction collection using the Vanquish HPLC and UHPLC systems <https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-72940-lc-fraction-collection-vanquish-tn72940-en.pdf>



Figure 10. Concentrate on work that matters. The Vanquish Analytical Purification LC system enables you to separate, purify and go for the next step in your process.

Learn more at thermofisher.com/LCpurification

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