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HPLC & UHPLC

Comparison of InVial and AboveVial mode in fraction collection on a Vanquish Analytical Purification LC system

Authors

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Keywords

Vanquish HPLC, fraction collector, InVial mode, AboveVial mode

Goals

Comparing the characteristics of the two collection modes: (i) **AboveVial** in which the collection needle is hovering over vials for minimizing contamination and (ii) **InVial** in which the collection needle can puncture through the sealed cap preventing solvent evaporation.

Introduction

The Thermo Scientific™ Vanquish™ Analytical Purification LC system provides high-resolution sample separation followed by precise sample purification, which is facilitated by the integrated Thermo Scientific™ Vanquish™ Fraction Collector (FC). The Vanquish FC comes with a 3-axis motion control, which enables the two collection modes—AboveVial and InVial—for flexible multi-application purposes (Figure 1).[1]

AboveVial mode provides low cross-contamination of fractions due to the non-wetted needle and the fast time of transferring to an adjacent vial (<0.75 s). The adjustable needle height enables the use of a wide variety of sample containers from 384 or 96 well plates to racks holding 2–10 mL vials. InVial mode enables sample collection from the bottom of the container (just 3 mm from the bottom) to avoid sample splashing during droplet formation. Notably, the collection needle can puncture sealed caps like the Thermo Scientific™ Vanquish™ Autosampler, thereby reducing the risk of sample evaporation.

In this technical note, five compounds were employed as standards, and gradient elution was used to evaluate the two collection modes. To be noted are risks associated with InVial collection and AboveVial collection. When collecting InVial, the Automatic (default) setting positions the needle 3 mm above the bottom of the vial. Therefore, the risk is that the fill level in the collecting vessel reaches the collection needle and this could result in the contamination to the outside of the collection needle. This can be avoided by adjusting the InVial needle height below the top level of the collection vessel but above the expected fill level of the liquid inside the collection vessel. When collecting AboveVial, the Automatic (default) setting positions the collection needle 2 mm above the specified vial or plate height. This is a safe setting for the needle when it moves along the collection path above the collection vessel. The risk associated with this collection mode is that various well plate or vial manufacturers have different well plate or vial heights and this could result in a collision of the collection needle with the side of the collection vessel. To be safe, test the needle height before beginning the fraction collection as described in the technical note: Principles of fraction collection using the Vanquish HPLC and UHPLC systems[1].

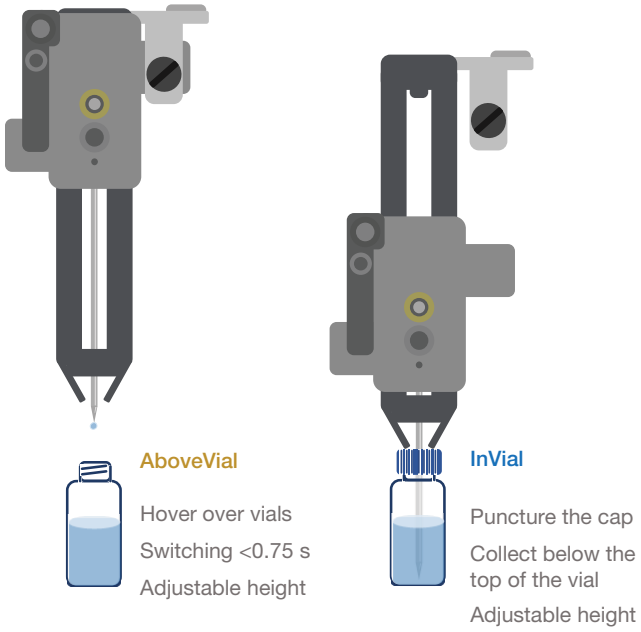


Figure 1. Two fractionation modes: AboveVial and InVial

Experimental

Chemicals

Acetonitrile (ACN, P/N 51101) was supplied by Fisher Scientific (USA). High purity standards (uracil, acetanilide, benzophenone, hexanophenone, and octanophenone) from Sigma-Aldrich were used without further purification. Ultrapure water (18.2 MΩ·cm) was produced in-house by a Milli-Q™ EQ 7008 (France). To obtain relatively consistent absorbance, the standards were accurately weighed and diluted with 80% (v/v) ACN in water to 5 mmol/L.

Instrument configuration

The Vanquish Analytical Purification LC system included a pump, autosampler, column compartment, diode array detector (DAD), and fraction collector, all controlled by the Thermo Scientific™ Chromeleon™ Chromatography Data System 7.3.1 and newer. Refer to Table 1 for configuration details.

Table 1. The configuration of the Vanquish Analytical Purification LC system

Description	Part number
System Base Vanquish Core	VC-S01-A-02
Vanquish Quaternary Pump C	VC-P20-A-01
Vanquish Split Sampler CT	VC-A12-A-02
Vanquish Column Compartment C	VC-C10-A-03
Column, Thermo Scientific™ Accucore™ C18, 150 × 4.6 mm, 2.6 µm	17126-154630
Vanquish Diode Array Detector CG	VC-D11-A-01
Vanquish Flow Cell, Bio, 2.5 µL, 7 mm, 120 bar	6083.0550
Vanquish Integral Fraction Collector FT	VF-F20-A-01
Delay Capillary for Time-based Fractionation, 0.10 × 350 mm	6042.2340
Vial, 2 mL Screw 9 mm Transparent Glass	6PSV9-1PSS
Cap Screw, 9 mm, Blue PP with Silicone/Red PTFE Septa	6PSC9ST1R

HPLC separation conditions and instrument settings

The typical gradient program is presented in Table 2. The instrument parameters are summarized in Table 3. Considering that raising the temperature will increase the volatility of solvent, especially for organic phase, the chamber temperature of the fraction collector was set at 40 °C to demonstrate the impact of evaporation in an extreme situation.

Table 2. Gradient elution profile

No	Time (min)	A % (H_2O)	B % (ACN)	Curve
Equilibration stage	-3.0	75%	25%	5
1	0.0	75%	25%	5
2	8.5	5	95%	3
3	10.0	5	95%	-

Table 3. Vanquish Analytical Purification system parameters

Module	Content	Parameters
Quaternary pump	Flow rate	1.0 mL/min
Autosampler	Injection volume	10 µL
Column compartment	Temperature	40 °C
DAD	Data collection rate	10 Hz
	Wavelength	254 nm
Fraction collector	Temperature	40 °C
	Collection mode	By time
	Needle positioning mode	InVial or AboveVial
	Wash needle	Both, 30 s
	Flush	On
	Start position	By Custom Variable
Collection timetable	Peak 1	1.120–1.340 min
	Peak 2	2.490–2.710 min
	Peak 3	4.990–5.210 min
	Peak 4	6.250–6.470 min
	Peak 5	7.950–8.170 min

Setting up an instrument method using the Vanquish Fraction Collector in Chromeleon CDS is simple and user friendly. A step-by-step instrument method wizard walks the user through each parameter required for this experiment. With the supplement of a simulation, the user can visualize based on a selected chromatogram a hypothetical collection. Also, the additional custom column to be added by the user in the sequence table to define the custom start position makes for a very useful tool for fraction collection flexibility.[2]

Results and discussion

UHPLC method for separation of standards

Due to the retention time shifts having a negative impact on fraction recovery, six replicates of five standards were performed to prove system repeatability, as shown in Figure 2. The RSDs of retention times were less than 0.03%, which is critical for further fraction collection (Table 4).

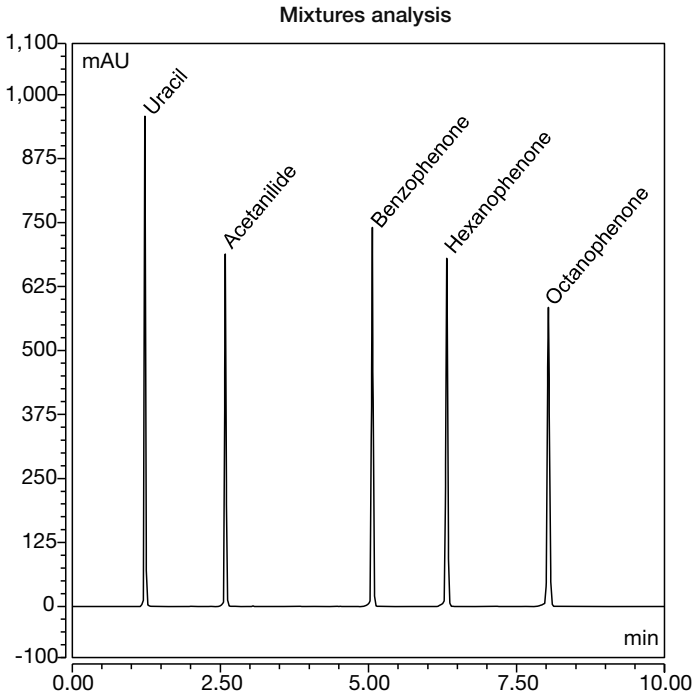
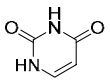
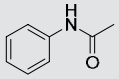
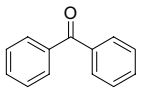
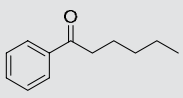
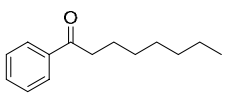


Figure 2. A typical chromatogram of the five mixed standards at 5 mmol/L

Table 4. Compound, elution solvent, and repeatability, n=6

Name	Structure	ACN % (Estimated)	Ret. time (min) RSD%	Area (mAU*min) RSD%	Height (mAU) RSD%
Uracil		25%	1.228 0.0%	22.448 0.19%	957.22 0.20%
Acetanilide		52%	2.258 0.02%	18.5161 0.20%	688.09 0.27%
Benzophenone		73%	5.065 0.01%	23.0942 0.17%	740.59 0.20%
Hexanophenon		80%	6.325 0.02%	25.5189 0.17%	681.32 0.18%
Octanophenone		88%	8.043 0.03%	25.0975 0.21%	585.40 0.13%

Re-analysis of the collected fraction

Based on the UHPLC system and conditions described in Table 2 and Table 3, the solution of five mixed standards was fractionated twice by applying the two collection modes, respectively: AboveVial (hover over vials) and InVial (fill from bottom with cap piercing capability) (Figure 3A). The collected fractions (n=10) were transferred to the autosampler immediately for re-analysis. The resulting chromatograms, presented in Figures 3B and 3C, show that the five standards exhibited single sharp peaks, and no significant differences between the two collection modes were observed. The zoomed baselines of the purified standards, as displayed in Figure 4A, demonstrate that there was no measurable carryover from previous peaks after flushing the collection needle.

To demonstrate the evaporation effects in the thermally controlled FC, fractionation was performed in an extreme situation (40 °C) with both collection modes. The sample containers with caps (InVial Collection) and without caps (AboveVial Collection) were stored in the FC module for an additional 4 hours. The five fractions with two collection modes were then re-analyzed, and significant differences were observed between the two collection modes. Due to cap sealing, evaporation was minimized and the peaks of the InVial mode were similar to those of the original immediate re-injections. The peak areas increased slightly, ranging from 100.7% to 101.8%. However, for the AboveVial mode, re-injection peak areas increased significantly compared to the cap sealed samples (Figure 4B). Furthermore, response increased with increasing retention time. This is likely due to the higher organic content in the collected fractions by the gradient elution resulting in increased evaporation during storage. The estimated acetonitrile content of the elution composition based on the retention time is referenced in Table 4. The apparent concentrations of the AboveVial collected standards were increased to 138.7%, 146.1%, 192.7%, 194.2%, and even 201.8% respectively, after 4 h storage at 40 °C.

Solvent evaporation

By weighing the collected solution, significant weight loss of sample solution was observed at the temperature of 40 °C in AboveVial mode, as shown in Figure 5A. In addition, because of the gradient elution, the later eluted components with higher organic phase ratio displayed marked weight decreases of 52%. In contrast, for the InVial mode, Figure 5B, the collected solution had no significant reduction in weight (only -1.6–-3.0%).

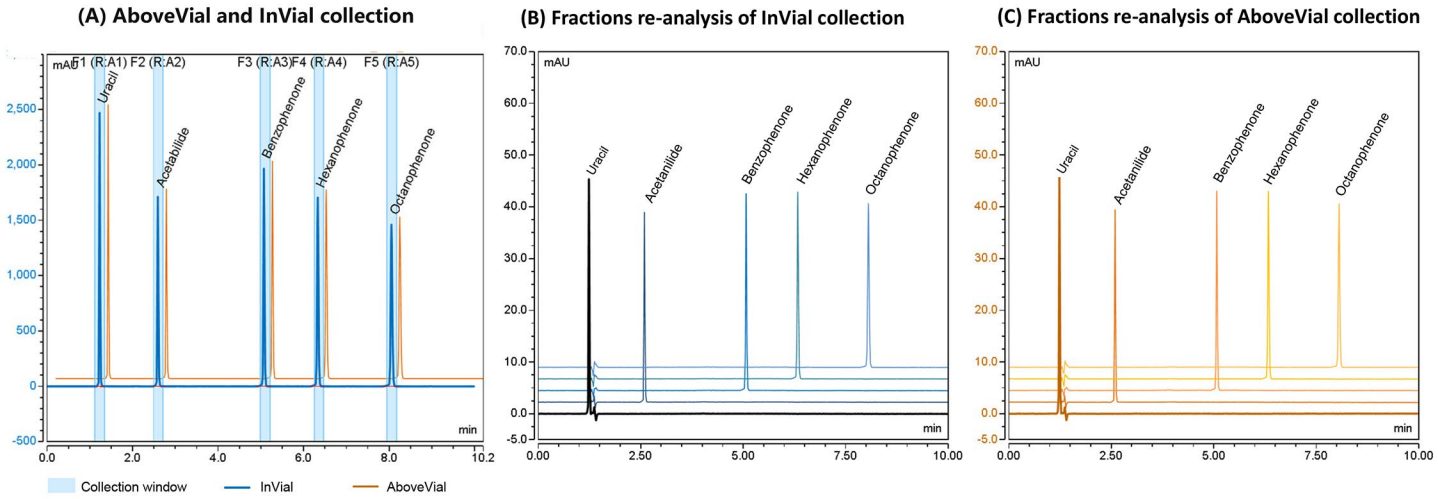


Figure 3. (A) The overlaid chromatograms in the same separation conditions with different collection modes, along with the the instant re-analysis results of purified standards by (B) InVial mode and (C) AboveVial mode

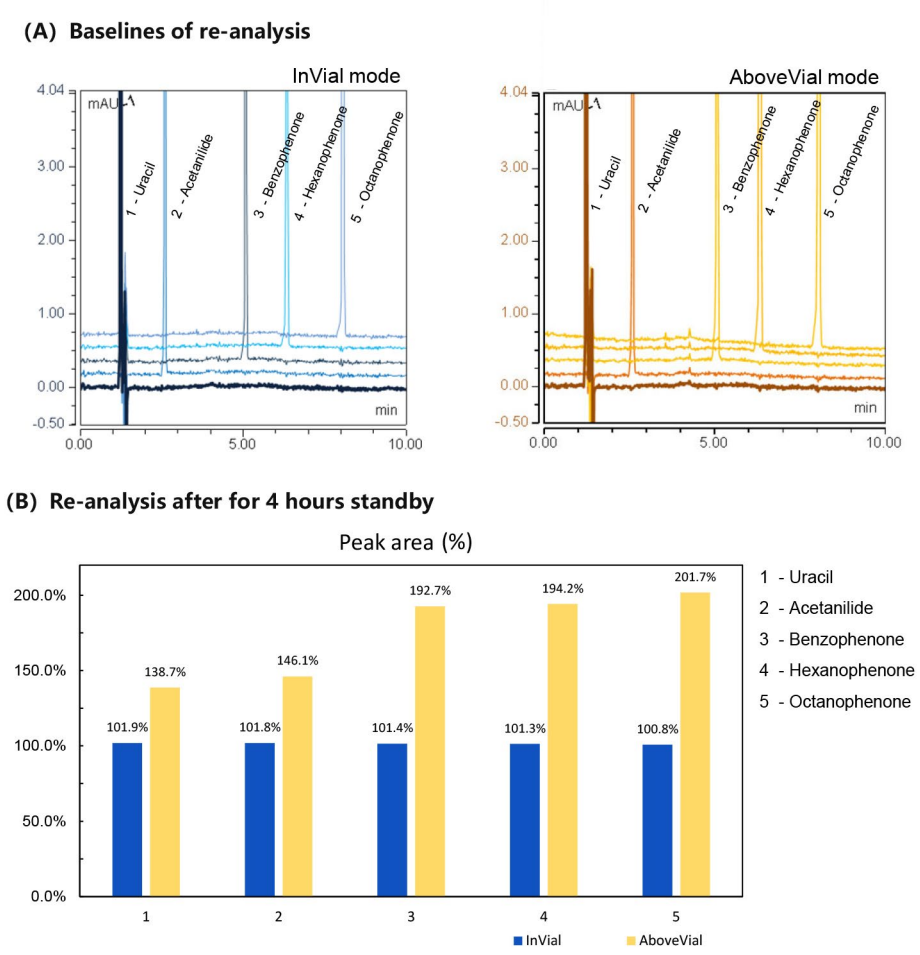


Figure 4. The zoomed baselines of purified standards demonstrate the high effectiveness of the fraction collector's ability to isolate the pure compounds with no measurable peak-to-peak carry-over (A) and comparison of re-analysis after 4 h storage at 40 °C (B)

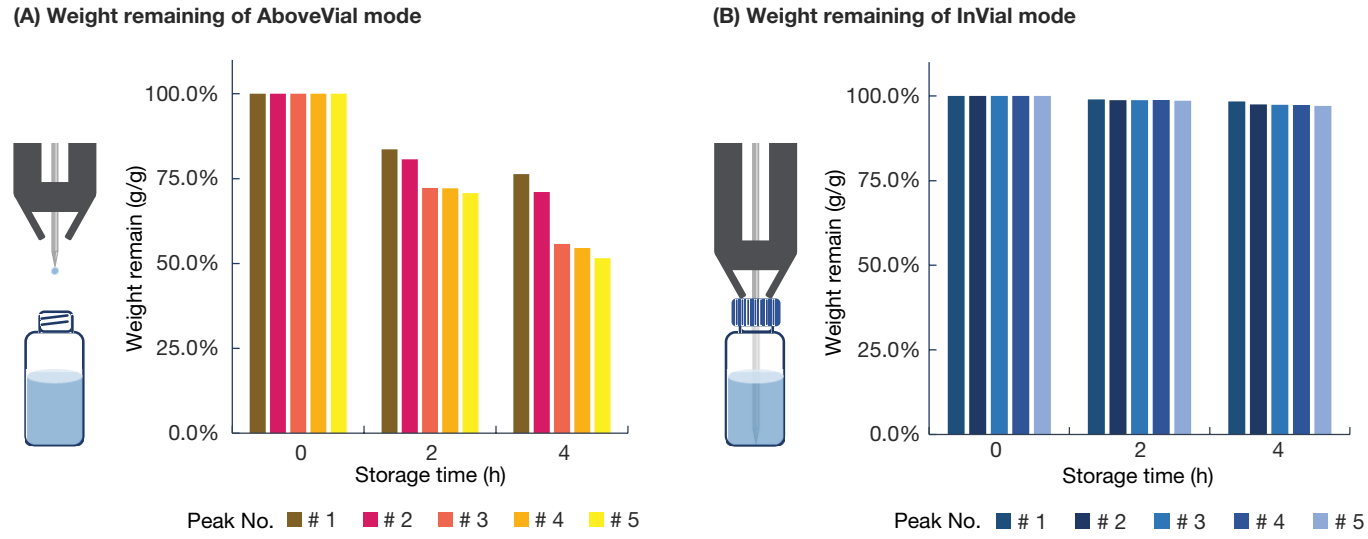


Figure 5. The weight remaining of cap opened (A) and sealed vials (B)

Conclusions

The Thermo Scientific Vanquish Fraction Collector controlled by Chromeleon CDS enables excellent fractionation, providing purer samples for further research. The two collection modes of AboveVial and InVial can be adopted for numerous applications with low cross contamination. While the AboveVial collection mode enables continuous fractionation with the fastest transition from vessel to vessel, the InVial mode offers the advantage of the exact positioning height of the needle within the vial to minimize the risk of any splashing even at higher flow rates. Moreover, combining the cap piercing capability and adjustable needle height, the collection function of filling from bottom effectively reduces volatile gradient solvent evaporation during fraction collection.

References

1. Koehler, D; Fabel, S.; Acworth, I.; Shen, G. Thermo Scientific Technical Note 72940: Principles of fraction collection using the Vanquish HPLC and UHPLC systems, **2022.** https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-72940-lc-fraction-collection-vanquish-tn72940-en.pdf
2. Shi, L; Li, Y; Liu, Y; Gao, L; Wang H; Yan Y; Wu, Y; Ran L; Jin, Y; Acworth, I; Koehler, D; Fabel, S. Thermo Scientific Technical Note 002751: Setting the start position for flexible fraction collection by using custom variables in Chromeleon CDS, 2024. https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-002751-hplc-vanquish-uhplc-fraction-collector-tn002751-na-en.pdf

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