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Principles of fraction collection using the Vanquish UHPLC systems

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Introduction

The principle of liquid fraction collection is an evolving science whose tools have developed into automated sample introduction, precise liquid chromatographic instrumentation, integrated software control, and the fraction collector apparatus itself.

Fraction collection is the process of capturing the eluting fluid in vessels based on a liquid chromatographic separation. One measures the presence and concentration of desired analytes via detection methods, ranging from UV detectors to mass spectrometers. These detectors in combination with the chromatographic data system (CDS) are used to determine the fraction window, either based on the detector signal or on the retention time.

This document focuses on analytical scale fraction collection (0.1 mL/min – 5 mL/min), the Thermo Scientific™ Chromeleon™ CDS software settings, inherent factors to be considered for best translation of chromatographic resolution to fraction purity, and the supporting UHPLC instrumentation.

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Fraction collection instrumentation and delay volume determination

The goal is to achieve the isolation of a target species or the segregation of impurities. The resolution achieved by the column needs to be reflected in the detector as well as in the fractions. This 1:1:1 ratio of chromatographic resolution to detection response to fractionation precision is an ideal. These are subject to diffusion, Eddy currents, and mechanical obstacles. A further obstacle in fraction collection is scaling up. This process is nonlinear and each application range possess its own character: nano, analytical, semi-preparatory, preparatory, and industrial scales in which the analytical application range is addressed here.

The majority of fractionation configurations are a setup of an HPLC system combined with a fraction collection device between the detector and the waste container. The fluidic pathway follows the standard HPLC conduit beginning at the solvent source delivering eluent with the pump through the autosampler, separating chromatographically on the column, and sending a signal response from the detector. The bridge capillary connects the detector with the final module: the fraction collector. A waste capillary is then attached to the fraction collector discarding the unnecessary eluent (Figure 1).

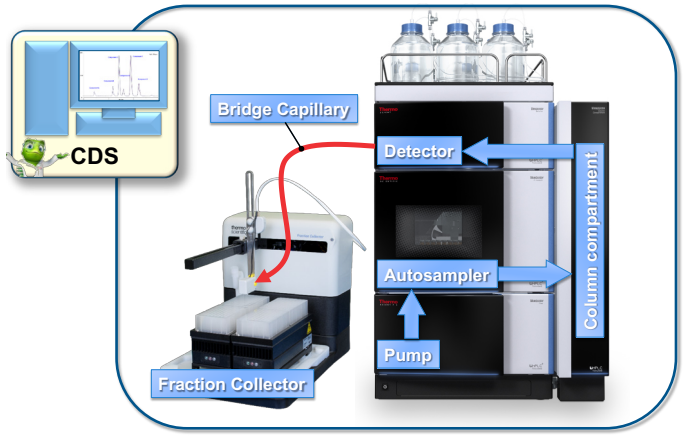


Figure 1. Typical configuration for fraction collection. The fluidic pathway is indicated and the CDS connects the entire setup communicating with the LC system to trigger fractions on the fraction collector.

The Thermo Scientific™ Fraction Collector F was used to provide the portioning of the fluidic volume to an XY defined location for a specified time range. Thermo Fisher Scientific offers two fraction collector options: the Fraction Collector F and the Thermo Scientific™ Fraction Collector FT. A third fraction collector option is available, though compatible only with the Thermo Scientific™ UltiMate™ 3000 portfolio and will not be discussed here. The Fraction Collector F is the base model delivered with a 1.0 mm ID drop-former configuration. The Fraction Collector FT has Peltier cooling elements to chill fractions to 4 ˚C and a 0.4 mm drop-former configuration designed for analytical flow rates. Both fraction collectors can have their drop-former kits modified post-purchase.

Any fraction collector has the ultimate goal to transfer a detector signal as accurately similar to the elution profile in the fractions as possible, but this ideal is never achieved due a delay volume between the detector and the collection vessels (Figure 2).

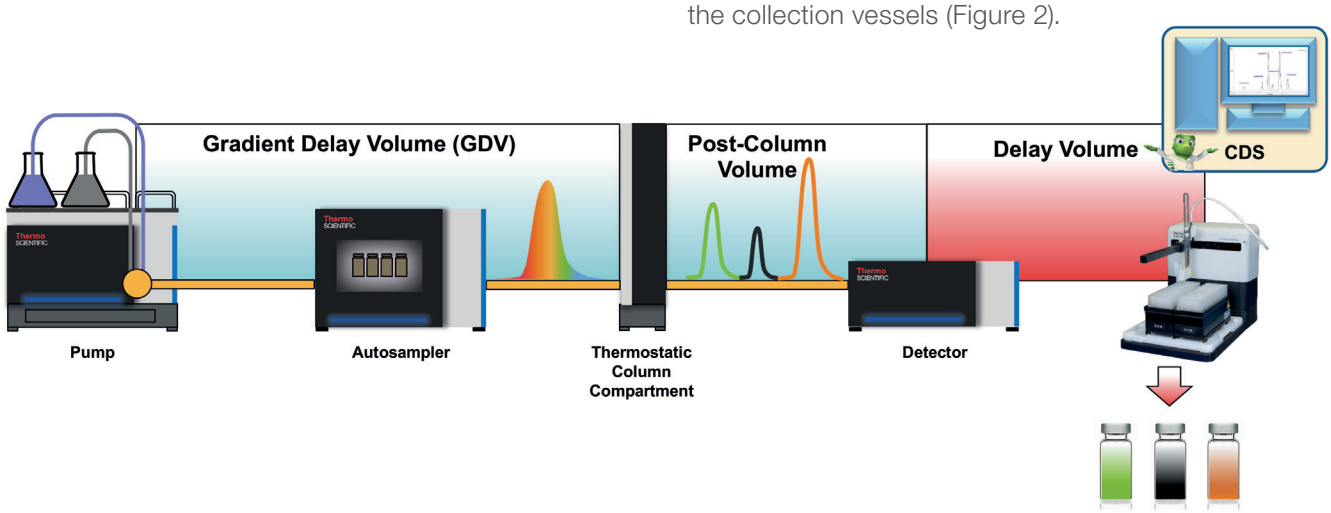


Figure 2. The delay volume is represented by the volume after the detector and before the collection vessel. This volume is entered in the CDS.

These are the factors contributing to the delay:

1. The bridge capillary is the connecting line between detector and the fraction collector's diverter valve 'sample inlet' port (Figure 3). The bridge capillary, diverter valve, and drop-former provide an important value called the delay volume. This volume needs to be accounted for by the CDS and according to the flow rate, a delay time is calculated. If this value is incorrect then peaks could potentially be distributed into the wrong fractions compared to the recording software. This volume contributes significantly to the dispersion between the detector and the fractions themselves.

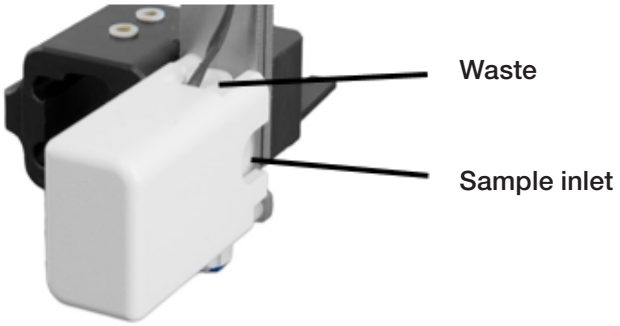


Figure 3. Diverter valve and connections: the bridge capillary outlet is screwed tight on the diverter valve via a nut and ferrule. Similarly, a waste line can be attached to the top of the diverter valve.

2. The diverter valve is solely responsible for redirecting the flow between a waste position and a collection position. Its fluidic volume will contribute to the delay.

   In the Fraction Collector F/FT, this diverter valve contains an L-joint, which connects sample inlet and waste or sample inlet and drop-former. The technical goal of the diverter valve is to switch quickly, to provide as little delay volume as possible, and to be positioned as close as possible to the absolute end of the LC fluidic pathway.

3. The drop-former is the final physical contributer to the fractioning resolution and can vary from a fused-silica capillary contributing a few fractions of a microliter to a spigot & hose setup for preparatory scales possessing several milliliters (Figure 4). Besides the additional volume, the surface tension of the mobile phase will generate a unique volume. The higher the organic content is, the lower the viscosity and surface tension, and therefore the smaller the resulting droplet volume is:[1]

   Droplet Volume ~ Viscosity ~ Surface Tension

   The Fraction Collector F/FT has the option between two drop-formers:

   - 0.4 mm i.d. drop-former = 1.3 µL
   - 1.0 mm i.d. drop-former = 4.8 µL

   This volume also contributes to the overall delay volume.

Figure 4. Displayed is the 1.0 mm drop-former delivered with the Fraction Collector F. There are two drop-former configurations: 0.4 mm drop-former with corresponding diverter valve and 1.0 mm drop-former with corresponding diverter valve.

4. The arm movement delivers the fluidics to an XY-plane position allowing the eluting solution to be distributed among vessels. Ideally, the arm movement is quick to its desired location and positions itself in the correct location. When increasing switching times of the diverter valve or increasing the time/distance moving from one collection vessel to the next, there is increased delay time and its corresponding volume with respect to the flow rate.

5. The CDS converts the detector signal into fractionation arm movement and diverter valve switching. Furthermore, the software is responsible for the collection mode definition. This means the software takes either peak parameters or timed parameters and triggers the fractioning arm movement. Factors such as the delay volume, peak detection settings, and timed collection settings have a direct influence on the delay time (and corresponding volume) of the chromatogram to elution profile in the resulting fractions.

Most of the factors contributing to the delay volume are given by the applied fraction collector. The bridge capillary can be optimized for the required flow rate range. Below are recommendations for analytical flow fractionation bridge capillaries with the corresponding Chromeleon CDS delay settings. Please note the *Derivative Step* and *Chromeleon CDS Delay Volume* to be entered in the CDS software as seen in Table 1.

Table 1. Recommended capillaries and Chromeleon CDS settings for given application flow rate ranges

Capillary Color	Capillary Ordered Length	Actual Length (±0.5 cm)	Compatible Flow Rate Range (mL/min)	Derivative Step Value (s)	Chromeleon CDS 7.2 Delay Volume (µL)
Red (125 µm i.d.)	152.4 cm (5 ft)	157.5 cm (5.17 ft)	0.5–0.8	0.02	55
Blue (250 µm i.d.)	152.4 cm (5 ft)	157.5 cm (5.17 ft)	0.5–1.2	0.02	86
Orange (500 µm i.d.)	152.4 cm (5 ft)	157.5 cm (5.17 ft)	1.2–5.0	0.02	334

Fraction collection modes

Fraction collection can be achieved either based on a time program or a peak recognition program (Figure 5). The selection of an appropriate collection mode depends on the chromatographic conditions, i.e., resolution of the separated compounds.

Table 2 gives recommendations for the selection of the fractionation mode.

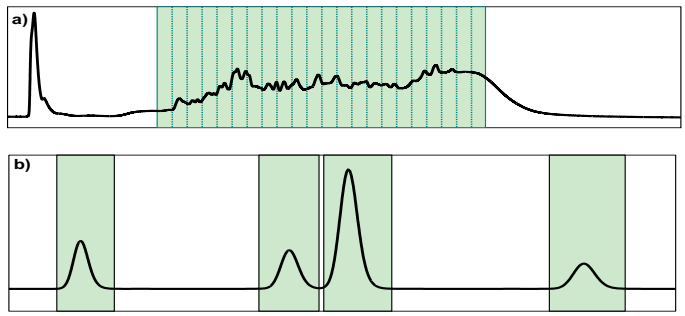


Figure 5. The chromatograms represent a) Collect by Time and b) Collect by Peak

Table 2. Recommendations for when to use peak-based fractionation and when to use time-base fractionation

Chromatographic condition	Time-based	Peak-based
Known stable retention times	X	
Known stable peak shape	X	
Unknown retention times		X
Peak shoulders		X
High resolution		X
Low resolution	X	
Very complex samples	X	

Software settings

The Chromeleon 7.2 CDS was used to perform the fraction collection. Chromeleon CDS provides a step-by-step wizard for method creation. The following explanation goes into the details of each fractionation parameter but will forego the other LC module parameters.

The most important software-related parameters include:

1. The collection channel – whether it be UV detection or another detection method, setting the channel correctly is essential. If one collects by peak and an incorrect absorption wavelength is set as the collecting channel, then it is possible to lose much if not all the desired analytes.

2. Selection of the appropriate fractionation mode (Table 2).

3. The delay volume – this setting is critical to be correctly entered (Table 1). If set too high, the desired analytes will be collected in either earlier-than-intended vessels or drained to waste entirely. If set too low, the desired analytes will reside in later fractions when correlating the fractions with the source chromatogram.

4. Automatic fraction pooling – this needs to be activated if it is desired to start the fraction collection of every sample run in the same initial vial. If deactivated, Chromeleon CDS remembers the last collection position from a run and then continues with the next collection position upon the next run.

5. The arm movement mode – the user can decide between a more self-explanatory collection progression with *SawVertical* or the more precise *Vertical* movement scheme. When the fraction collection arm reaches the end of a row in *SawVertical* mode, the arm has a longer distance to travel to the first position in the next row versus in *Vertical* mode.

6. The collection mode: *Continue* vs. *Interrupt* - in Chromeleon CDS, one can set the diverter valve to continue the flow through the drop former between fractions or send the flow to waste between fractions. At lower flow rates, it is recommended to set the diverter valve on *Continue* as the exiting liquid forms drops, minimizing loss due to the liquid missing the fraction vessel and guaranteeing no material is lost to the drain position and thus to waste.

The settings in the software can contribute to unnecessary loss, and at analytical flow rates this can make for a larger impact on the final fraction recovery. For example, in *SawVertical* mode, the diverter valve switches to drain when changing from the end of one row to the beginning of the next. The collection order may be easier to follow on the tray though sending the flow to waste during arm transfer is a source of loss. Additionally, if *Interrupt* collection mode is selected, the fluid is diverted quickly to waste between each fraction contributing to a loss in recovery. Though at higher flow rates and larger peak volumes, this is perhaps negligible. Therefore, for best analytical flow results, it is recommended to use the combination of *Continue* collection mode with the *Vertical* movement scheme for minimized loss.

For step-by-step guidance of the fraction collection section of the method editor, please refer to **Appendix A**.

Variables and considerations for fraction collection

Delay – Given the hardware setup (Figure 1), the traditional configuration places a detector before the fraction collector. Using a UV detector as an example, an analyte enters the flow cell generating an electronic signal whose intensity is based on the concentration of the sample and absorbing capacity of the analyte. This electronic signal is interpreted by the software to meet conditions of threshold and slope. When the software determines a condition has been met, the software sends a trigger to the fraction collector. During this short time window after the analyte has exited the flow cell it continues through the bridge capillary until it reaches the drop-former. The time or volume it takes the analyte to reach a fraction vessel from the flow cell is the delay time. Naturally, given a specified flow rate, this delay time can be translated into delay volume (Figure 6). This value as volume or time is entered into Chromeleon CDS to trigger the diverter valve to switch from the position “drain” to “collect” after the signal trigger is received and the delay has been reached.

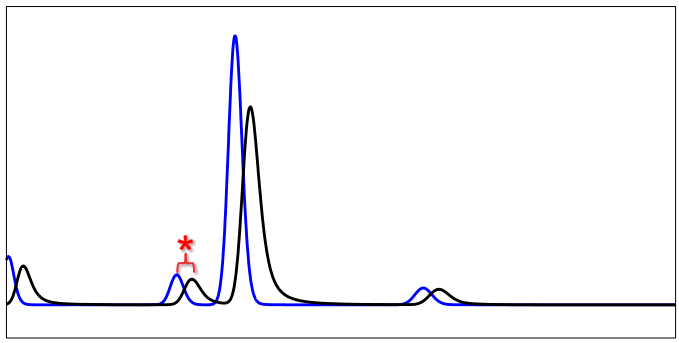
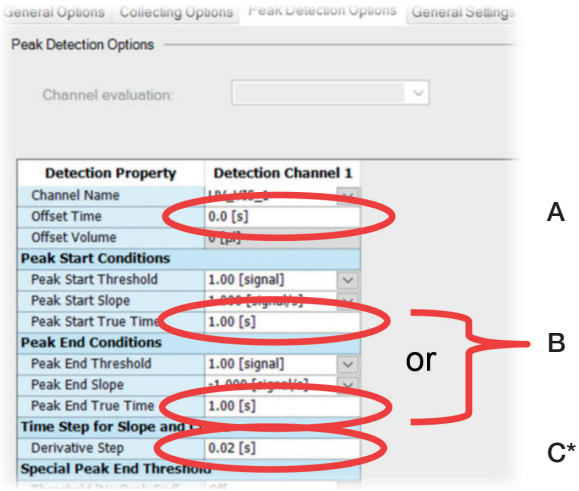


Figure 6. The delay volume (*) is the difference in time between the chromatogram from the detector (in blue) and the resulting elution chromatogram delivered at the tip of the drop-former (in black) caused by the GDV addition of the diverter valve, drop-former, and bridge capillary.

Chromeleon CDS has a specific formula to evaluate the delay volume or time compatibility in that the sequence ready check will provide an error if the delay volume or time is not compatible with the method. There needs to be time for not only Chromeleon CDS but also the detector to respond to slopes and thresholds. Additionally, the delay time factor of 3 s is programmed into the method wizard as a computational buffer. The entire delay time is a combination of the physical delay from detector to fraction collector, settings in Chromeleon CDS, and the calculating time. Therefore, the software calculates the minimum delay time:

Minimum delay time = OffsetTime + DerivativeStep + Max(PeakStartTrueTime or PeakEndTrueTime) + 3 s

The *Delay Time* or corresponding *Delay Volume* must be greater than or equal to this computation (Figure 7).



A+B+C+3 = 4.1 s

***Adjusted from 1.0 (s)**

Figure 7. During peak-based fractionation, these parameters add to the total delay time or corresponding delay volume. Chromeleon CDS adds an additional 3 s for mass directed fractionation computational time.

Lastly, 25 µL was added as an additional volume, which was observed when an elution profile was re-analyzed to correct for a volume inherent in the diverter valve.

Dispersion – This occurs immediately after the sample is drawn in the syringe needle and whose impact can be measured in resolution and peak broadening. The capillaries, column, flow cell, and fraction collector are the largest contributors of dispersion. Specifically, the importance of measuring the impact of dispersion between detector and fraction collector is shown in Figure 8.

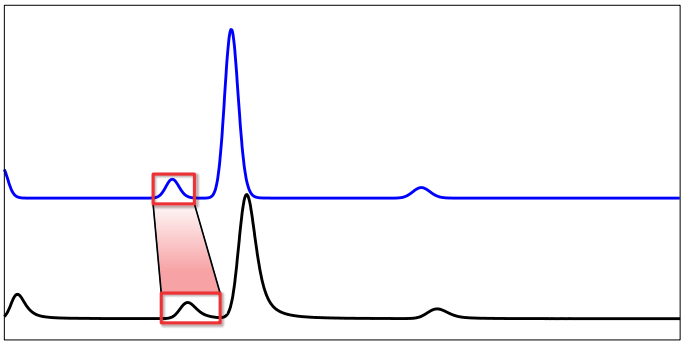


Figure 8. Dispersion sources influence the original chromatogram (in blue) resulting in the elucidating chromatogram (black), exemplifying peak broadening due to the bridge capillary, diverter valve, and drop-former.

Factors between the detector and collection vessel contributing to dispersion are capillary length, capillary diameter, detector flow cell, diverter valve, and drop-former. The flow cell is selected based on the analyte concentrations and peak volumes, but once selected, the influences the flow cell has on dispersion is constant. The drop-former can be selected to have a 0.4 mm i.d. or a 1.0 mm i.d. Below 2 mL/min, the 0.4 mm i.d. drop former is recommended to minimize dispersion and carry-over. The contribution of the diverter valve on the dispersion is also constant.

The largest impact the user has on the dispersion between fraction collector and detector is the bridge capillary length and inner diameter.[2] Since an increase in the inner diameter of the capillary system increases exponentially the diffusion, the best results can be met with the smallest i.d. bridge capillary possible. This is represented by the *Golay equation*.[3] Therefore it is important to install the smallest i.d. bridge capillary. The length of the capillary is then matched to reflect the required delay time determined by the equation: OffsetTime + DerivativeStep + Max(PeakStartTrueTime or PeakEndTrueTime) + 3 s. The limitation of the bridge capillary i.d. on the system is the backpressure, which exponentially increases with the i.d. and thus influences the choice for a complementary backpressure-resistant flow cell. Therefore, the backpressure caused by the bridge capillary is measured before installing it into the fluidic path. Given these influences, Table 1 outlines the match between application flow rate and corresponding specific bridge capillary. These pre-cut capillaries are available in the optional Fraction Collector F/FT 0.4 mm drop former kit.

When contaminations from previous fractions are observed while collecting by time, this is an effect of dispersion or improper delay settings and not carry-over. This effect can also be observed when the detecting chromatogram displays baseline and the re-analyzed fractions contain trace amounts of the previous peak.

This factor of dispersion principle is shown in Figure 9. Fraction 7 exhibits a very slight presence of the compound found in Fraction 2. This exemplifies the diffusion effects resulting in increased tailing after the detector due to the bridge capillary and diverter valve volumes. To counter this effect, a thinner bridge capillary whose resulting backpressure does not damage the flow cell is recommended (suggested configurations located in Table 1).

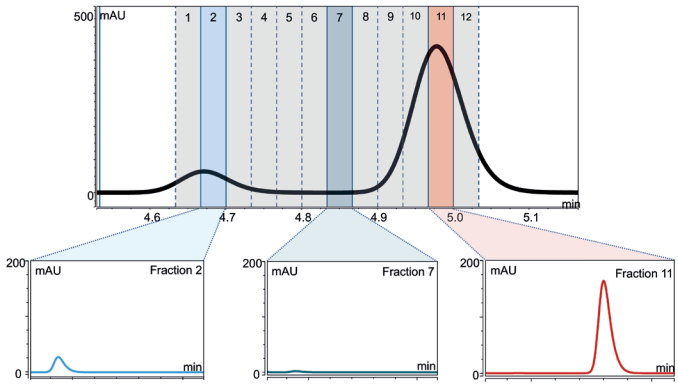


Figure 9. The above time-base fractionation was subsequently re-analyzed showing that in Fraction 7 (green) a tailing-effect caused by diffusion. The re-analysis of Fraction 2 (blue) shows a pure compound. In the source chromatogram, one observes a baseline in Fraction 7 though in the resulting re-analysis, there is a trace amount of the compound seen in Fraction 2.

Carry-over – There is an inherent flaw in all fraction collectors in the volume between the tip of the drop-former and the diverter valve exit. This volume is excluded from the fluidic pathway when the valve is switched to drain. If this drop-former volume contains product from a tailing peak and then the drop-former is switched to drain, this volume packet in the drop-former will co-elute with the detected and eluted next peak. This factor of carry-over is experienced only when collecting by peak and contributes to poorer resolution between fractions. This effect can be observed in the elution and re-analysis in Figure 10.

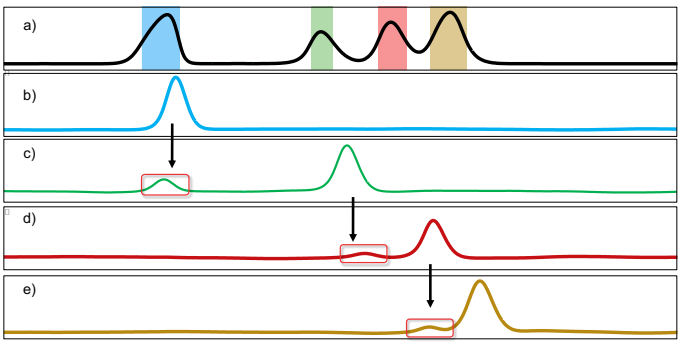


Figure 10. The source chromatogram for the fractionation using a 0.4 mm drop former kit represented by a) is subsequently re-analyzed in b), c), and d), showing prior peak carry-over due to the drop-former volume not being flushed between peaks. This is only applicable during peak-based fractionation.

Experimental

Time-based fractionation

Using the information listed above, an alkylphenone mixture was fractionated in a time-based fashion to exemplify the characteristics of the described LC configuration listed in Table 3. This results in the chromatogram shown in Figure 11. The fractions were then re-analyzed and the resulting elution profile was compared to the source chromatogram in Figure 12.

Table 3. Experimental application details: Collect by Time

System	
Thermo Scientific™ Vanquish™ Quaternary Pump F (VF-P20-A)	
Thermo Scientific™ Vanquish™ Split Sampler FT (VF-A10-A-02)	
Thermo Scientific™ Vanquish™ Column Compartment H (VH-C10-A-02)	
Thermo Scientific™ Vanquish™ Variable Wavelength Detector (VF-D40-A)	
Fraction Collector F (VF-F11-A-01)	
7 mm Semi-Micro Flow Cell (P/N 6077.0360)	
Chromeleon CDS, Feature Release 7.2.8	
Reagents	
Ultra-pure lab water, 18.2 MΩ·cm at 25 °C	
Acetonitrile, Optima™ LC/MS (Fisher Scientific P/N A955-212)	
Methanol, Optima LC/MS (Fisher Scientific P/N A456-212)	
Fractioning conditions	
Sample	100 µg/mL uracil, acetanilide, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone, octanophenone
Column	Thermo Scientific™ Acclaim™ 120 C18, 3 µm, 4.6 x 150 mm (P/N 059133)
Eluent	30% methanol in water isocratic
Flow Rate	0.8 mL/min
Run Time	20 minutes
Pressure	220 bar
Temperature	40 °C
Injection	1.0 µL
Detection	254 nm, 20 Hz, 0.2 s response time, 4 nm bandwidth
Fractionation Settings	Collect by peak, 2 second fractions, vertical collection, 0.02 s Derivative Step, 55 µL delay volume, 127 µm x 157.2 cm bridge capillary
Re-analysis conditions	
Column	Thermo Scientific™ Accucore™ Vanquish™ C18+, 1.5 µm, 2.1 x 100 mm (P/N 17326-102130)
Mobile Phase	A – water B – acetonitrile Isocratic @ 45% B
Flow Rate	0.5 mL/min
Run Time	3 min
Pressure	885 bar
Temperature	40 °C
Injection	1.0 µL
Detection	254 nm, 20 Hz, 0.2 s response time, 4 nm bandwidth

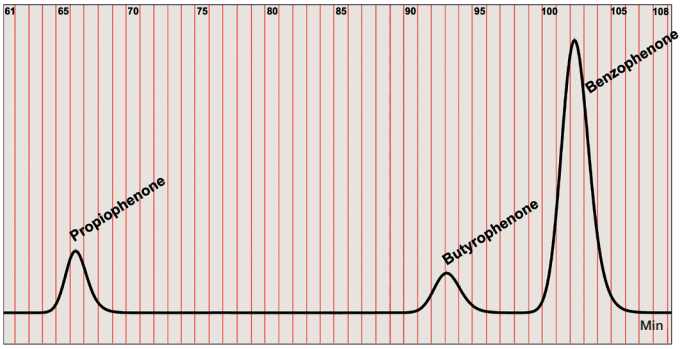


Figure 11. Based on the software parameters, the above chromatogram was collected in time-based fractions. The re-analysis profile can be seen in Figure 12.

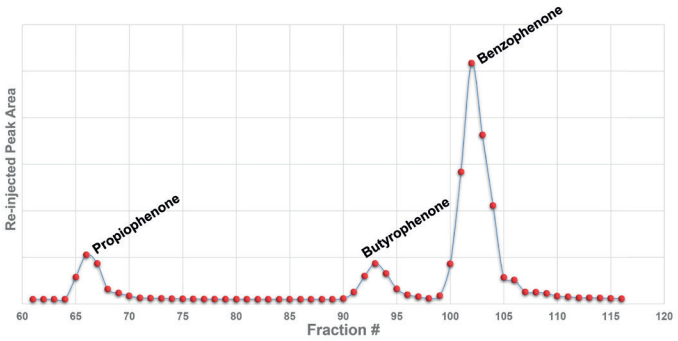


Figure 12. The re-analysis of the time-based elucidation represented in Figure 11. The areas of each fraction were plotted against the number of the fraction.

Peak-based fractionation

Similarly, an alkylphenone mixture was fractionated in a peak-based fashion to exemplify the characteristics of the described LC configuration listed in Table 4. This results in the chromatogram and subsequent re-analysis shown in Figure 13.

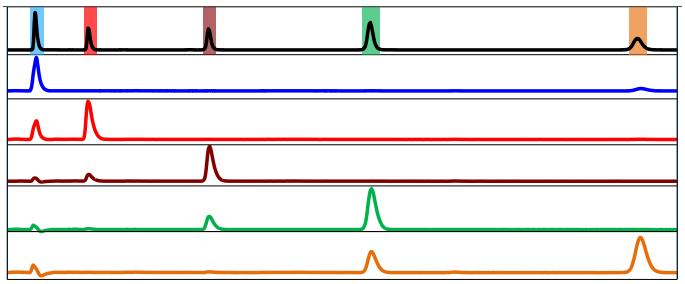


Figure 13. Based on the software parameters, the top chromatogram was eluted with peak-based fractionation. The subsequent chromatograms represent the re-analysis of each fraction.

Table 4. Experimental application details: Collect by Peak

System	
Vanquish Quaternary Pump F (VF-P20-A)	
Vanquish Split Sampler FT (VF-A10-A-02)	
Vanquish Column Compartment H (VH-C10-A-02)	
Vanquish Variable Wavelength Detector (VF-D40-A)	
Fraction Collector F (VF-F11-A-01)	
7 mm Semi-Micro Flow Cell (P/N 6077.0360)	
Chromeleon CDS, Feature Release 7.2.8	
Reagents	
Ultra-pure lab water, 18.2 MΩ·cm at 25 ˚C	
Acetonitrile, Optima LC/MS (Fisher Scientific P/N A955-212)	
Fractioning conditions	
Sample	40 µg/mL uracil, 200 µg/mL 4-nitroaniline, 1003 µg/mL methyl benzoate, 3510 µg/mL ethoxybenzene, 5512 µg/mL o-xylene
Column	Thermo Scientific Acclaim 120 C18, 3 µm, 4.6 x 150 mm (P/N 059133)
Eluent	80% acetonitrile in water isocratic
Flow Rate	0.75 mL/min
Run Time	10 min
Pressure	147 bar
Temperature	40 ˚C
Injection	5.0 µL
Detection	254 nm, 20 Hz, 0.2 s response time, 4 nm bandwidth
Fractionation Settings	Collect by peak, Peak Start Threshold 3 mAU, Peak Start Slope 1.0 mAU/s, Derivative Step 0.02 s, 0.3 mL Max tube volume, vertical collection, 55 µL delay volume, 127 µm x 157.2 cm bridge capillary
Re-analysis conditions	
Column	Thermo Scientific Accucore Vanquish C18+, 1.5 µm, 2.1 x 100 mm (P/N 17326-102130)
Mobile Phase	A – water B – acetonitrile Isocratic @ 45% B
Flow Rate	0.5 mL/min
Run Time	3 min
Pressure	885 bar
Temperature	40 ˚C
Injection	5.0 µL
Detection	254 nm, 20 Hz, 0.2 s response time, 4 nm bandwidth

Results and discussion

Time-based fractionation – When comparing the source chromatogram (Figure 11) and its resulting elution profile (Figure 12), one can draw a few conclusions regarding the efficacy of the fraction collector.

a. The peak in the source chromatogram for each of the components starts at Fraction 64 for propiophenone, Fraction 90 for butyrophenone, and Fraction 99 for benzophenone. This is reflected in the re-analysis of the eluted fractions where it was determined that the delay time or volume has been set correctly.

b. The propiophenone peak in the source chromatogram concludes in Fraction 69. In the re-analysis of the eluted fractions, Fraction 74 is the concluding fraction for propiophenone. This exemplifies slight tailing and the impact of dispersion caused by the diverter valve and bridge capillary. This depreciates the resolving power of the application and needs to be considered with barely to non-resolving peaks.

Peak-based fractionation – The re-analysis of the top chromatogram's fractions from Figure 13 shows a separation that relays an efficacy, considering the properties of the fraction collector and its properties, which mostly isolates each peak. Naturally, the carry-over characteristic of each fraction collector with peak-based fractionation influences the purity. This collection feature is useful for initial method development and good resolving peaks.

Conclusion

The efficacy of fractionation can be determined by comparing the sample chromatogram at the detector with the resulting elution profile achieved by re-analyzing the collected fractions. One would ideally observe that the resulting elution profile would match exactly the corresponding peaks of the sample chromatogram, but this is naturally hindered by fixed properties of each fraction collector. Diffusion, carry-over, software properties, and mechanical movement are all factors that contribute to this non-ideal performance. These factors can all be qualitatively determined to make up a composite of valuable fraction collector characteristics.

References

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Appendix A: Chromeleon CDS Fraction Collection wizard

1. Begin by creating a new instrument method and enter the pertinent information for the pump, autosampler, column compartment, and detector.

2. General options for fraction collection:

   a. **Off (do not collect at all):** This will defer all eluting solvent from the LC system through the waste line on the diverter valve.

   b. **Collect by peak:** This will trigger the collection based on chromatographic peak recognition parameters such as threshold, peak slope, etc. defined in the *Peak Detection* section.

   c. **Collect outside of peaks:** This is selected in only combination with *Collect by Peak* to collect the eluent in fractions before and after recognized peaks. There is the *Collecting Options* section to define either how much volume these between-peak fractions can possess or how much time for each fraction is allotted.

   d. **Collect by time:** This setting forces the user to set a time slice for each fraction. This parameter will override the *Max Tube Volume* in the *Collecting Options*.

   e. **Collection time frame:** The default condition for *Start Time* is *Start Run* to begin collection at the time of injection. If a time (in minutes) is specified, all eluent before this entered time goes to waste over the diverter valve. Subsequently, the default value for *End Time* is *End Run* and an entered time after the *Start Time* will eliminate any fraction collection after this specified time. *Append Range* adds a new range to the last entry row. *Insert Range* inserts a range above the selected row. *Delete Row* removes the range.

f. **Select injection:** This button will open a dialog box prompting the user to select an example chromatogram on which a simulated fractionation pattern will be presented. In the dialog box, one can navigate into other sequences, select different channels, and injection types.

3. Collection options

a. **Pump device:** This parameter defines which module is responsible for determining the flow and thus the corresponding volume in the collection vessels.

b. **Max tube volume:** This parameter is used in conjunction with *Collect by Peak* in the *General Options* and defines the maximum volume a collection vessel can contain.

c. **Max number of tubes per fraction:** This parameter is used in conjunction with *Collect by Peak* in the *General Options*. After the number of vessels entered in this field is reached for a specific peak being collected, the valve switches to drain and no further fractions of that specific peak will be collected.

d. **Total number of tubes:** This parameter defines the absolute number of tubes in the rack(s). After the total number of tubes has been reached in the unfinished run, the arm moves back to the first collection position and continues to collect at that initial position.

e. **Minimum time for tube change:** This parameter is used in conjunction with *Collect by Peak* in the *General Options* and overrides a tube change triggered by the *Peak Detection* options. This minimum time needs to be reached for the arm to move on to the next fraction whether or not the peak detection options trigger a tube change (Figure 14). This setting can also be used to avoid filling tubes with an insufficient volume when collecting fractions.

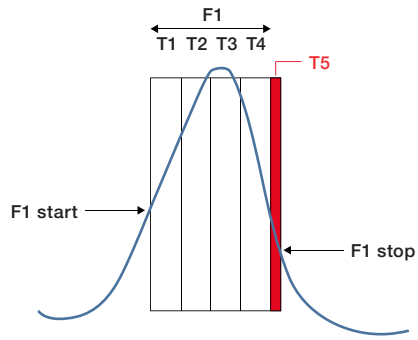

Figure 14. Avoid illogically small fractions by defining a minimum volume a fraction needs to have to proceed to the next fraction. F1 refers to Fraction 1 or the entire peak and T1–T5 refers to the individual tubes or fractions. One wants to avoid a small fraction due to Collect-by-Peak parameters represented by T5. To avoid this small fraction T5, set the *Minimum time* for tube change to a larger volume.

f. **Tube wrapping:** When this option is activated, the arm will move back to position 1 in a run after the end of the available vessels has been reached.

g. **Fraction pooling (reset the tube position for each run):** When this option is activated, the arm will move always to position 1 for subsequent runs. If this option is not selected, the subsequent run will continue to the position right after the last collected vessel from the prior run.

h. **Delay time:** This is the difference in time (seconds) when a peak elutes through the detector and arrives at the drop-former. Or it is the difference in time between which the CDS recognizes a collection trigger based on time or peak, and when the diverter valve in the fraction collector switches from drain to collect. It can either be specified in *Delay Time* or *Delay Volume* fields.

i. **Delay volume:** This is the difference in volume (µL) represented by the bridge capillary, diverter valve, and drop-former volumes. Since there is a minimum time necessary for the CDS to react to a peak, the corresponding volume can be calculated based on the flow rate. In Chromeleon CDS, the minimum time is represented by the equation: determined by the equation: *OffsetTime* + *DerivativeStep* + *Max(PeakStartTrueTime* or *PeakEndTrueTime)* + 3 s. This is the minimum volume necessary between which the CDS recognizes a peak or time collection trigger, the time the diverter valve positions itself correctly in space, and then switches from drain to collect. It can either be specified in *Delay Time* or *Delay Volume* fields.

4. Peak detection options – *The Peak Detection Options page is only available when the Collect by Peak option is selected.*

    a. **Show advanced settings** (located in the bottom left corner): when this box is checked, there are 11 further peak detection options available.

    b. **Preview** – Select injection: select a chromatogram on which a simulated fraction collection will be portrayed.

    c. **Channel evaluation:** When in the Instrument Configuration, the fraction collector's number of detection channels can be set here. The default value is one (1) channel so therefore the Channel Evaluation cannot be changed. When in the instrument configuration and the number of channels is 2 or more, one can select the channel to be used to trigger the fraction collector here.

    d. **Channel name:** Select in this field the specific wavelength at which the peak detection is to take place defined by the *Channel Name*. The listed *Channel Name* correlates to the defined channel in the *General Detections Options* of the instrument wizard.

    e. **Offset time:** *Advanced setting*. This entered time will be added to the total delay and is simply an additional delay factor in seconds between the *Peak Detection* trigger and the switch of the diverter valve from drain to collect. This option is only available if there is a *Delay time* specified in *Collection Options* and not a *Delay volume*.

    f. **Offset volume:** *Advanced setting*. This entered volume will be added to the total delay volume and is simply an additional delay factor in µL between the *Peak Detection* trigger and the switch of the diverter valve from drain to collect. This option is only available if there is a *Delay volume* specified in *Collection Options* and not a *Delay time*.

    g. **Peak start threshold:** The peak start triggers when the signal value exceeds this entered value and when the *Peak Start Slope* condition has been met. This value can span the range of the signal axis. This value can be also turned off to specify that peaks will be collected by the *Peak Start Slope* only.

    h. **Peak start slope:** The peak start triggers when this slope value is exceeded and the *Peak Start Threshold* condition has been met. The slope evaluation proceeds after the *Peak Start Threshold* signal has been reached. This value can also be turned off such that the peak start trigger will depend only on the *Peak Start Threshold*.

    i. **Peak start true time:** *Advanced setting*. This parameter sets a window of time in the chromatogram. During this time window, the relevant peak start conditions must be met so that the peak start can be triggered. This time window needs to be met to fully evaluate the peak start. The range is 0.0–4.0 s and directly influences the delay time. It accordingly influences the delay volume with the corresponding flow rate. A smaller value would be selected for smaller peak volumes.

    j. **Peak end threshold:** The peak end triggers when the signal value drops below this entered value after a peak maximum has been met. Unlike the peak start conditions, this value is independent of the *Peak End Slope*.

    k. **Peak end slope:** The peak end triggers when the slope value in (-) signal/s, exceeds the value entered. This peak evaluation occurs only above the signal defined by the *Peak End Threshold* and after a peak maximum.

    l. **Peak end true time:** *Advanced setting*. This parameter sets a window of time during the chromatography after a peak maximum condition has been met. During this time window, the relevant peak end conditions must be met so that the peak end can be triggered. The range for the time window is 0.0–4.0 s and directly influences the delay time. This time window needs to be met to fully evaluate the peak end. It accordingly influences the delay volume with the corresponding flow rate. A smaller value would be selected for smaller peak volumes.

    m. **Derivative step:** *Advanced setting*. During this interval, the difference in signal is evaluated so that its slope can be determined i.e. Peak Start Slope & Peak End Slope. The larger this value is the more noise will be filtered out and not collected. This range is 0.02–60.00 s and directly influences the delay time. It accordingly influences the delay volume with the corresponding flow rate.

n. **Threshold "no peak end":** *Advanced setting*. This value defines the end of a peak based on the precondition that a peak maximum has been met and the signal has fallen below the entered value. This value overrides any *Peak End Slope* trigger after a peak maximum has been reached. This function is used when a detector signal becomes saturated. The noise at saturation generates numerous *Peak End Slope* triggers and the goal is to continue the collection as one peak. Note: when the *Max Tube Volume* is reached, a new fraction vessel is chosen otherwise the peak is collected in one vessel until the *Threshold "No Peak End"* is met (Figure 15).

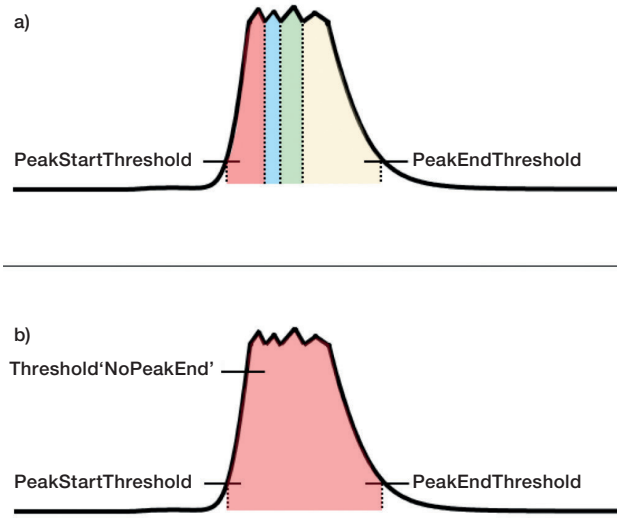

Figure 15. Set the *Threshold "No Peak End"* to eliminate subdivisions of an oversaturated peak. a) The slight valleys subdivide the peak at its saturation point into multiple fractions. b) Set the Threshold "NoPeakEnd" below the saturation point and the plateaued peak will be collected as one peak versus numerous slices. This is only applicable during peak based fractionation.

o. **Threshold "do not resolve":** *Advanced setting*. This feature overrides the Peak End Slope. It is used when the valley between two peaks does not reach the baseline and one wants to collect everything between two peaks. When this entered signal value is met after a peak maximum is detected, the next fraction will be selected only after the slope reaches 0.0 (Figure 16).

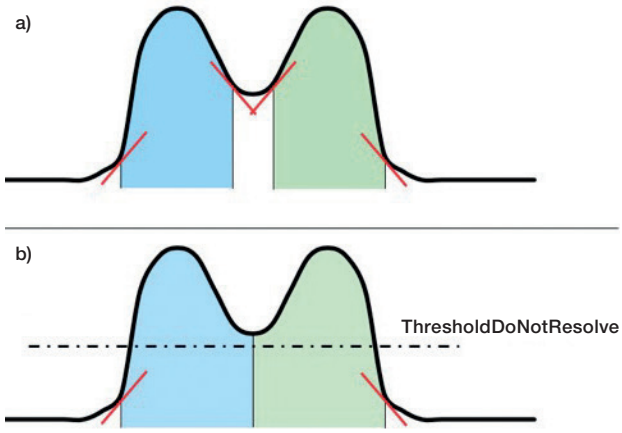

Figure 16. When two fractions do not resolve, there are two options. a) Two fractions generated with the standard begin slope and end slope settings. This generates a section between the unresolved peaks which does not collect due to the slope settings indicated in red. b) Set the Threshold "Do Not Resolve" parameter below the valley between two unresolved peaks to avoid an undesired subdivision in the valley. This is only applicable during peak based fractionation.

p. **Peak max slope:** *Advanced setting*. When this value is entered in (-) signal/s, the peak maximum will only be associated with a peak whose exit slope is less than the entered value. This avoids the splitting of peaks which have shouldering or long tailing. The greater the negative slope setting, the sharper the peaks need to fall off to have a defined peak maximum condition. The default value is "off". Note: for a peak that does not meet peak maximum conditions, there can be no peak end trigger. Thus, the peak will continue to be collected until the next peak start trigger has been issued.

q. **Peak max true time:** *Advanced setting*. This defines a time window (0.00–4.00 s) in which the peak maximum conditions must be met for there to be a peak maximum.

r. **Peak start curve:** *Advanced setting*. This threshold is used to detect shoulders on the front side of peaks whose unit is (signal/s^2). The condition can only be met after a peak start has been triggered but before a peak maximum has been defined. The higher the value entered the sharper the shoulder needs to be to be recognized as a separate fraction.

s. **Peak end curve:** *Advanced setting*. This threshold is used to detect shoulders on the tailing side of peaks whose unit is (signal/s^2). The condition can only be met after a peak start and a peak maximum have been triggered but before a peak end has been defined. The higher the value entered the sharper the shoulder needs to be to be recognized as a separate fraction.

t. **Baseline drift:** The *Baseline Drift* is used in combination with the *Baseline Offset* to compensate the signal value for drift and is a function of time with the unit signal/s. A calculated signal value is extrapolated from the equation:

Calculated Value = Signal Value – (BaselineOffset + Δt*BaselineDrift)

u. **Baseline offset:** The *Baseline Offset* is used together with the *Baseline Drift* to correct the signal value in order to compensate for drift. A calculated signal value is extrapolated from the equation:

Calculated Value = Signal Value – (BaselineOffset + Δt*BaselineDrift)

5. General settings

a. **Collect mode:** This setting determines how the fraction collector meanders when it reaches the end of a row. The choice is between *Vertical* or *SawVertical* (Figure 17).

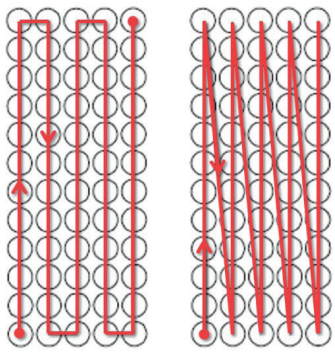

Figure 17. There is a choice between *Vertical* (left) and *SawVertical* (right) meandering schemes.

b. **Movement mode:** This setting determines if the diverter valve is switched to the drain position when moving from one collection position and the next. *Interrupt* switches the diverter valve to drain between fractions and *Continue* do not. This is exceptionally important when collecting in the *SawVertical* movement mode because Chromeleon CDS automatically requires the setting *Interrupt* to avoid contamination.

c. **Rack 1-6:** These settings allow the user to specify which collection rack is going to be used during fraction collection. When combining racks of different types, please refer to the Chromeleon CDS Help to determine which rack combinations are compatible. Below is a list of compatible collection vessel types:

- 250 mL bottles
- Well plates (96, deep and shallow)
- Vanquish Split Sampler sample rack: 12 mm OD vials (≤1.5 mL)
- Thermo Scientific™ UltiMate™ 3000 Well Plate Sampler sample rack: 12 mm OD vials, cylindrical (≤1.5 mL)
- UltiMate 3000 Well Plate Sampler sample rack: 15 mm OD vials (4 mL)
- UltiMate 3000 Well Plate Sampler sample rack: 22.5 mm OD vials (10 mL)
- Funnel for fractionation of unlimited volumes into appropriate vessels
- 30 mm (OD) x 100 mm (H) tubes (50 mL)
- 24 mm (OD) x 100 mm (H) tubes (30 mL)
- 20 mm (OD) x 100 mm (H) tubes (20 mL)
- 16 mm (OD) x 100 mm (H) tubes (14 mL)
- 13 mm (OD) x 100 mm (H) tubes (8 mL) (default configuration)
- 30 x 17 mm (OD) x 100 mm (H) tubes (15 mL)

d. **Automatically move to rinse position when sequence ends:** This defines at the end of the sequence if the fraction collection arm moves to the rinse position.

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