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Application Note

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Hipersep® Flowdrive Pilot System and Cyclojet Process Enables Intensified HPLC

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Abstract

The pharmaceutical manufacturing landscape has changed significantly over the past decade and is continuing to evolve rapidly. Producers have begun to wield new tools to achieve new efficiencies and deliver better medicines. Changes in the pharmaceutical industry are driving new manufacturing requirements, meaning that facilities must provide for:

- Complex molecules, multiple products and smaller batch sizes
- High temperature processes
- Flexible, easy to clean, compact equipment

Hipersep® Flowdrive Pilot is a new generation of HPLC Chromatography system based on these new trends such as higher regulatory constraints, system and process flexibility and reliable innovations to fit with complex purification (Peptides, Oligos, mRNA ...).

This application note highlights how Hipersep® Flowdrive Pilot and the Cyclojet process, a single-column sequential process significantly increases productivity, purity and yield, especially when the resolution of the batch process is limited. Cyclojet, also known as Steady State Recycling (SSR), recycles the non-resolved part of the chromatogram, injects the feed, and collects the purified products at each cycle. Compared to "normal elution," it makes better use of process time, solvent, and the stationary phase.

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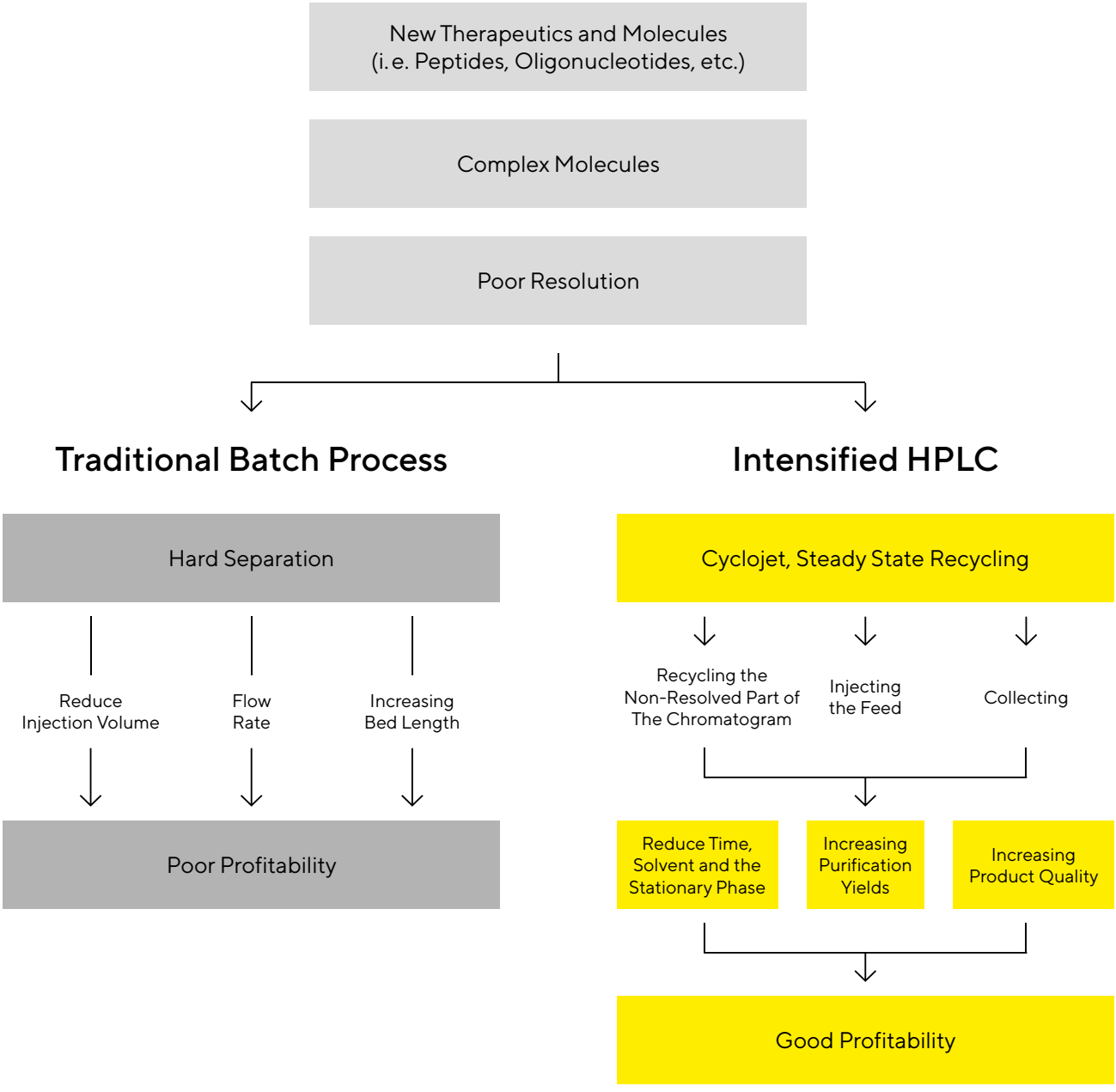
Introduction

The resolution of an elution is a quantitative measure of how well two elution peaks can be differentiated in a chromatographic separation. For some applications (e.g. Chiral separation) or complex multi-product separations (e.g. peptides, oligos), traditional chromatography in batch mode reveals poor resolution due to other products or contaminants very similar to the target product. Using these process conditions, it is difficult to isolate the target product in large quantities.

The industrial dimensioning of such a process is not economically and technically realistic. This leads producers to use different techniques to achieve the performance targets: reducing injection volume, reducing flowrate, increasing bed length ... All these solutions imply a loss of development and production time.

Figure 1: *The Purification of New and More Complex Molecules With Traditional Batch Processes Limits Profitability. The Cyclojet Process Can Be an Efficient Answer and Enables Intensified HPLC.*



Materials and Methods

Hipersep® Flowdrive Pilot is a a modular, compact and ergonomic system designed to minimize cross-contamination risk. It performs small-scale process development in the Lab on a 50 mm ID column at 100 mL/min and produces a certain amount of purified product in GMP environment on a 150 mm ID DAC column at 1,500 mL/min.

- Built-in flexibility with over 1,000 possible configurations that integrate with columns from 50 to 150 mm ID
- Minimize risk of cross contamination with a sanitary, easy-to-clean concept
- Save facility space with a compact and ergonomic design

In collaboration with a customer, we developed the Cyclojet process, also known as Steady State Recycling (SSR). This consists of recycling the non-resolved part of the chromatogram, injecting the feed and collecting the purified products in each cycle (Figures 2 and 3). This is a non-continuous cyclic recycling process that involves one or two columns. Compared to "normal elution," it makes better use of process time, solvent and the stationary phase. Purification Yields are also significantly increased, as well as product purities (sup. 95%). This is a proven process on an industrial scale to produce omega-3 fatty acids; Eicosapentaenoic acid (EPA), contained in fish oil. This complex purification mixture presents similar challenges to modern and complex peptides purifications. Combining Steady State Recycling (SSR) increases productivity, especially when the resolution of the batch process is limited.

Figure 2: *Typical Chromatogram for Cyclojet at the First Cycle.*

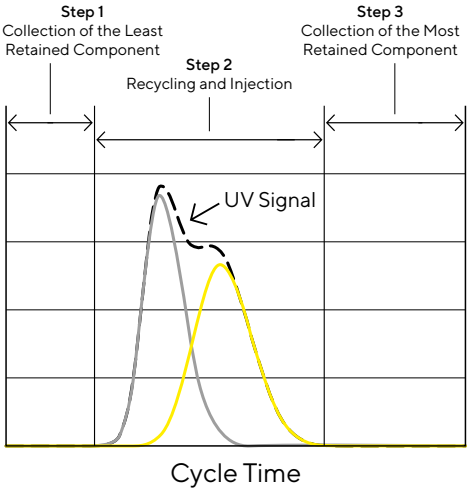
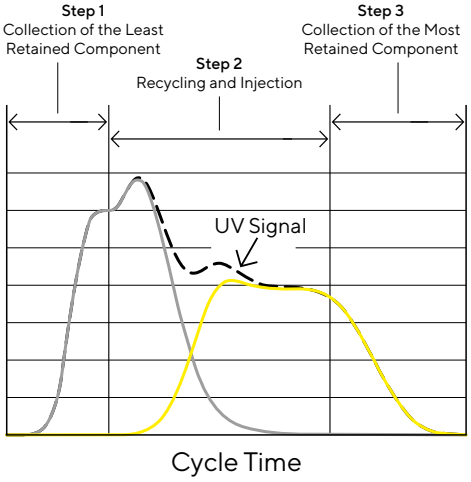


Figure 3: *Typical Chromatogram for Cyclojet at Steady State.*



Results

Case Study 1: Example with Chiral Separation

The case study presented is the purification of 1, 2, 3, 4 tetra-hydro 1 naphtol (tetralol). The racemic mixture to purify is selected using unfavorable operating conditions (low selectivity and poor resolution).

The experimental study has been performed using Hipersep® Flowdrive Pilot system and Hipersep® Prochrom 50 column.

Table 1: *Description of Operating Process Conditions*

Description	Operating Process Conditions
Column (ID × L)	50 × 277 mm
Stationary phase	Chiralpak AD, 20µm
Flowrate	317 (mL/min)
Temperature	27 °C
Feed concentration	40 (g/L)
Eluents	n-Heptane 95% v IsoPropanol 5% v Trifluoro acetic acid 0.2% v

It appears (Figures 4 and 5) that on a classical elution process, the resolution being so weak, many parameters would have to be modified and tested in order to reach both good purity and high yield such as:

- Decrease injected volume: useless in this case as even on analytical injection, peaks are not resolved.
- Reduce the flowrate to increase the number of plates: increase the cycle time and therefore reduce the production.
- Increase bed length: it takes time to prepare a new column and requires the use of additional amount of the stationary phase.
- Use a classical recycling method, or perform "peak shaving": will increase cycle time and therefore will increase the production time.

All these solutions imply a loss of both development time and production.

Figure 4: *Analytical Injections on Preparative Column*

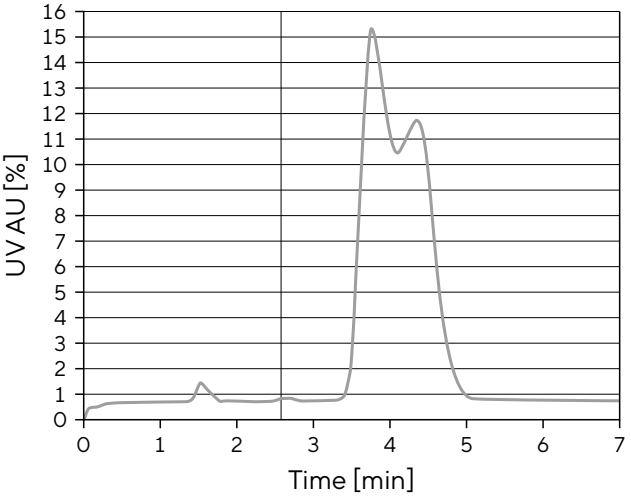
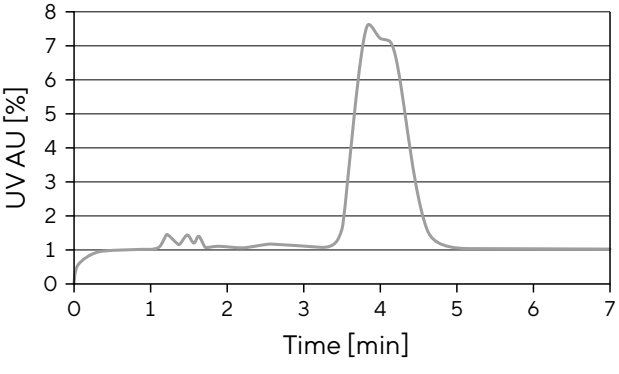


Figure 5: *Overloaded Injections on Preparative Column*



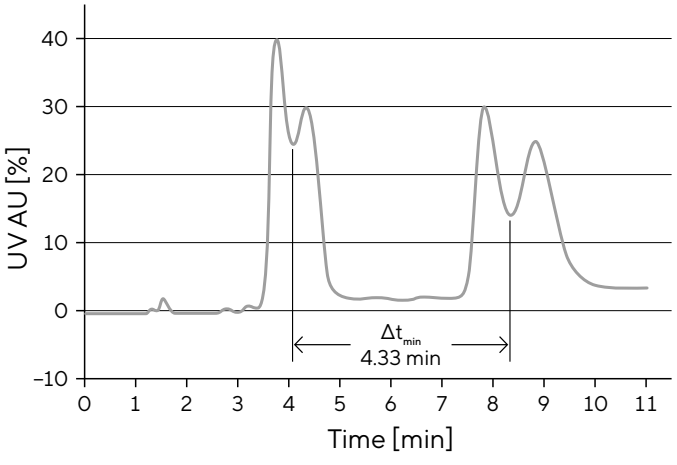
A simple experimental method allows determining Cyclojet process conditions, especially the cycle time and the injected quantity.

The following method was utilized:

- Perform an overloaded injection, such that the peaks are not baseline resolved on the column. Recycle the complete chromatogram back onto the column. If the chromatogram of the recycled injection is not baseline resolved, decrease the injected quantity and repeat the experiment. Adjust the injected quantity until baseline resolution is more acceptable (see figure 6).
- Measure the time between the minima of the initial injection and the recycled injection (as shown in figure 6).

The cycle time for the Cyclojet operation will be between 0.9 and 1 × Δt_{min}

Figure 6: *Raw Estimation of Injected Volume and Cycle Time*

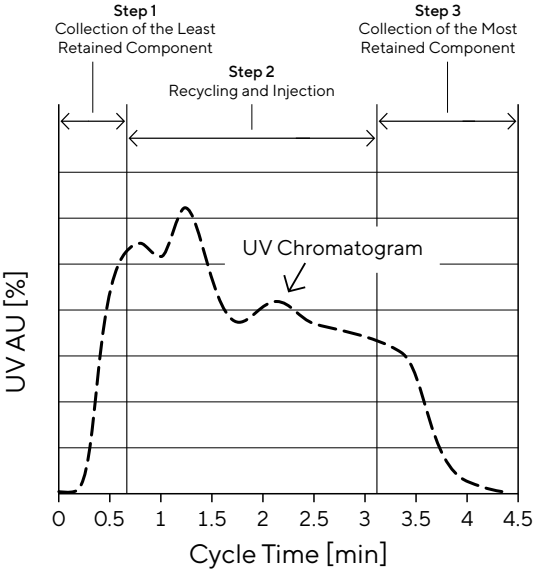


Using this method and few modifications of sequence parameters, the following performances have been reached:

Table 2: *Description of Operating Process Conditions*

Description	Performance
Total cycle time (min)	4.32
Recipe sequences	Step 1: from 0 to 0.66 minutes Step 2: from 0.66 to 3.13 minutes Step 3: from 3.13 to 4.32 minutes
Raffinate purity	99.5%
Extract purity	96.2%
Production	0.3 kg_{RAF}/ kg_{CSP}/day
Eluent consumption	980 L/kg_{FEED}

Figure 7: *Cyclojet UV Profile*



Results

Case Study 2: Process Concept With Multi-Components Product

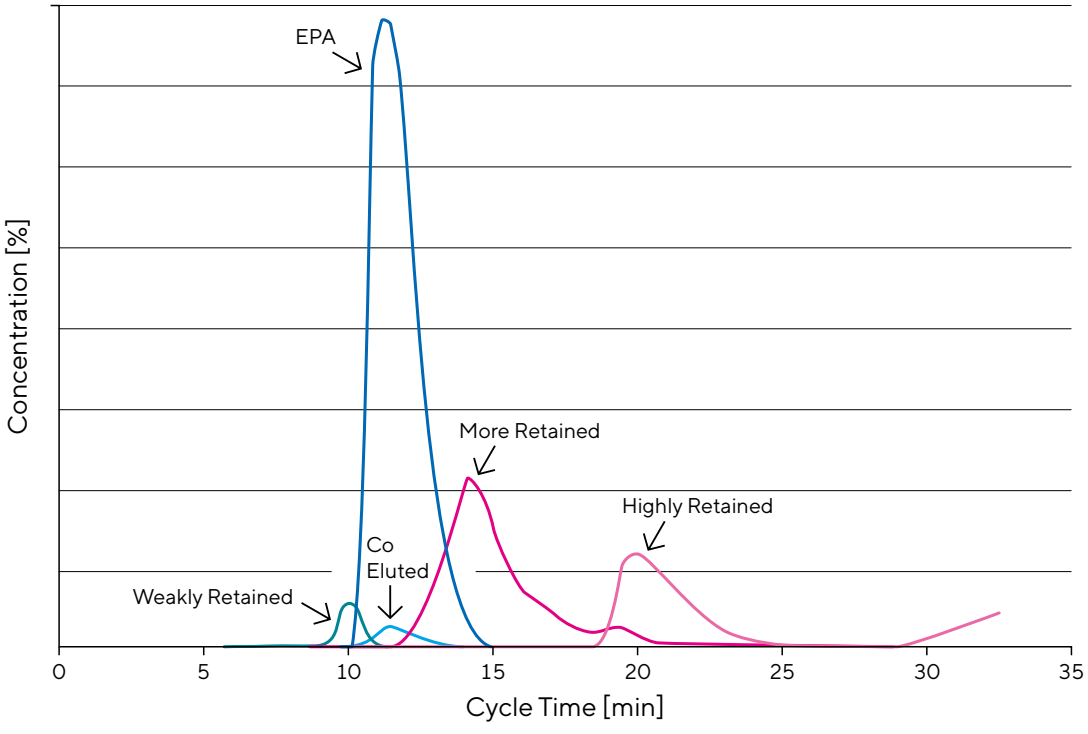
The case study presented is the patented purification of an omega-3 fatty acid (EP2883860, EP3079788, EP3079787, EP2801604), the Eicosapentaenoic acid (EPA), contained in fish oil. This complex mixture was selected to reflect another major application for HPLC chromatography as peptide purifications.

The preparative chromatogram obtained by peak reconstruction reveals peaks with an extremely wide range of retention times as shown in figure 8.

Data

- Weakly retained impurities (WRI) are eluting close to EPA. Their purification is extremely difficult using a batch process and so they are to be removed by means of optimized process.
- Icosapent ethyl: Target product.
- More retained impurities are also reported their purification to be extremely difficult with a conventional process so it also has to be removed by means of optimized process.
- Highly retained impurities (HRI): the strong difference in retention times makes this separation easy, potentially achievable using an HPLC process. The main related issue is the use of large amounts of mobile phases.

Figure 8: *Peak Reconstruction Profiles for Feed Fish Oil*



The chromatogram reveals a poor resolution with traditional batch process due to WRI and MRI which are very close to the target product. The separation makes impossible the isolation of EPA in large quantities by using these process conditions. The industrial dimensioning of such a process is economically and technically not realistic. Due to poor performances, the operation cost of large industrial units coupled with a low production rate lower the profit and increase the risks for pharmaceutical companies.

Multi compound products and low-resolution separation are the key parameters to identifying good candidates for the Cyclojet process. As the Cyclojet process is based on binary separation (raffinate/extract), the isolation of one compound comprising less retained impurities and more retained impurities must be done by using two Cyclojet process steps. The concept is to remove the more retained impurities during the first Cyclojet step and the less retained impurities during the second Cyclojet step. Achieving a highly productive preliminary treatment is accomplished by removing highly retained impurities.

Figure 9 illustrates the distribution of EPA and MRI on the first step. EPA is collected in the raffinate, MRI are collected in the extract, both with high yields and purities. On the second step, EPA is collected in the extract with a high yield.

Figure 9: *Cut Illustrations of the Two Cyclojet Process Steps*

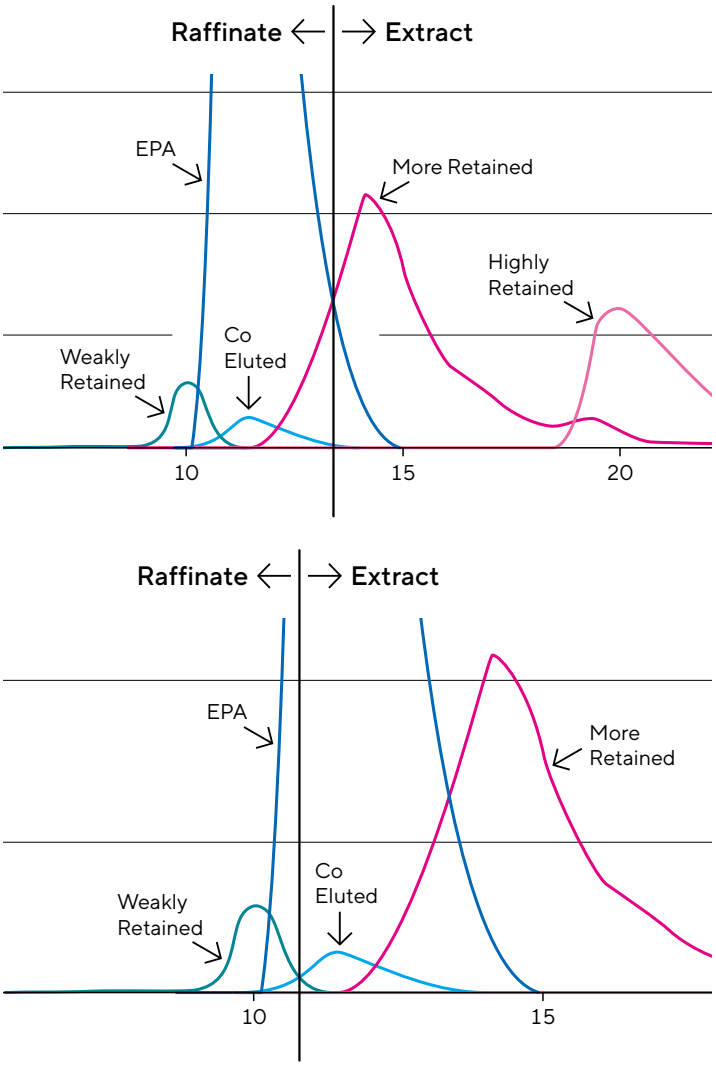


Table 3: *Summarized Performance Results Obtained on the Purification of EPA Contained in Fish Oil and by Using the Concept of Two Steps Cyclojet Process*

Steps	Performance
Feed fish oil compositions after pre-treament	EPA purity >70% (GC area)
Final product compositions after first Cyclojet step	EPA purity >92% (GC area)
Final product compositions after second Cyclojet step	EPA purity >97% (GC area)

Conclusion

The results of this case study demonstrate that for some difficult separations, such as chiral or peptide chromatography, the Cyclojet process – and in particular the two-step Cyclojet concept – can increase productivity, purity and yield to make the process beneficial to pharmaceutical companies.

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