APPLICATION NOTE

How Hipersep® Pilot HPLC system

answers new requirements in the pharma industry

novasep
passion & smart processes

The pharmaceutical manufacturing landscape has changed notably over the past decade and is continuing to evolve rapidly.

Pharmaceutical manufacturers have begun to wield some new tools, nailing down new efficiencies and better drugs in the process.

The development and production of pharmaceutical drugs are driving new requirements and change the way pharma producers are selecting and using their equipment.

- More complex molecules and increased regulatories as a need for greater cleanability, sanitary design and separation performances.
- Time to market and price pressure drives outsourced production, multiproducts factories and batch size reduction. Then push for flexible and compact equipment with a quick set-up.

Novasep has developed his new HPLC pilot skid based on these new trends such as higher regulation constraints, system and process flexibility and reliable innovations to fit with complex purification (peptides, Oligos...).

This application note highlights how Novasep Hipersep® Pilot is answering these requirements, surrounded by customer evaluation data.

Jérôme Chevalier, product manager at Novasep

Hipersep® Pilot is a High Performance Liquid Chromatography skid dedicated for the development & validation of pharmaceutical purification processes.

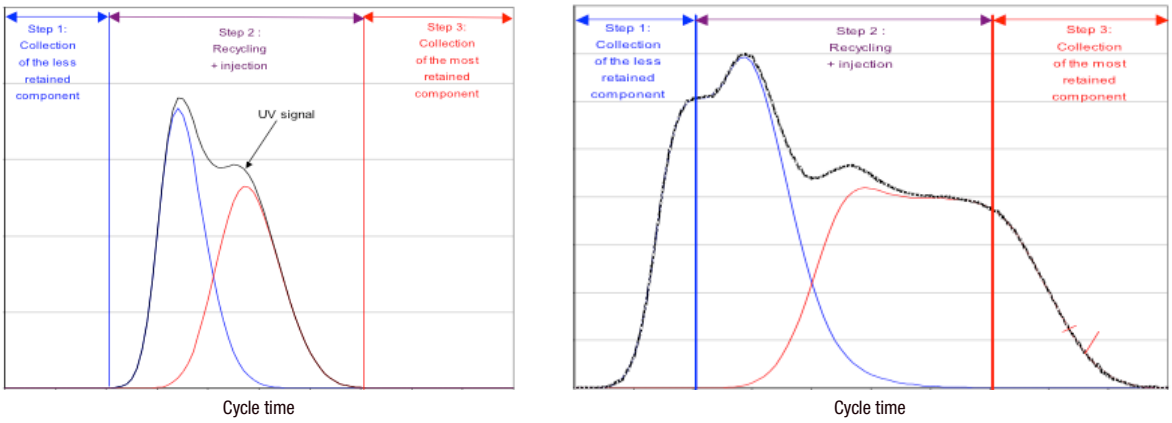
This purification system is specially designed to answer new requirements in the Pharmaceutical industry such as higher regulation constraints, system & process flexibility and reliable innovations. **Hipersep® Pilot** performs small scale process development in the Lab on a 50mmID column at 100mL/min and produces a certain amount of purified product in GMP environment on a 150mmID DAC column at 1500mL/min. A unique sanitary design and a modular approach allow user to perform in an effective way multi-product usage with adapted functionalities to perfectly fit with these needs.

Need for better resolution...
with Cyclojet for difficult separation

The resolution of an elution is a quantitative measure of how well two elution peaks can be differentiated in a chromatographic separation. Resolutions for several applications, such as peptide, oligonucleotides and chiral separation are too low, leading chromatographer to use different techniques in order to reach purification performance. These solutions such as reducing injection volume, reducing flowrate, increasing bed length... are done to the detriment of cost.

Presentation of the Cyclojet process

The Cyclojet is an innovative chromatography process to increase productivity, particularly when the resolution of the batch process is limited. Cyclojet also known as Steady State Recycling (SSR) consists in recycling the non-resolved part of the chromatogram, injecting the feed and collecting the purified products at each cycle. This process is a non-continuous cyclic recycling process that involves one or two columns. Compared to "normal elution", it makes better use of time, solvent and stationary phase. Yields of purifications will also be significantly increased.



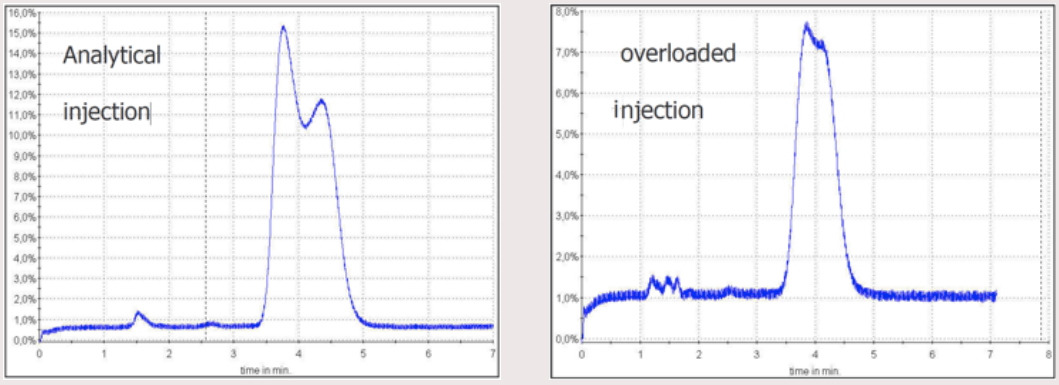
Typical chromatogram for Cyclojet at the first cycle (on left) and at steady state (on the right)

CASE STUDY 1
EXAMPLE WITH CHIRAL SEPARATION

The case study presented is the purification of 1,2,3,4 tetrahydro 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 Novasep Hipersep® system and Prochrom® LC50 DAC column. The operating conditions are listed in the following table:

DESCRIPTION	OPERATING PROCESS CONDITIONS
Column (IDxL) mm	50 x 277
Stationary phase	Chiralpak AD, 20µm
Flowrate (mL/min)	317
Temperature (°C)	27
Feed concentration (g/L)	40
Eluents (v/v)	n-Heptane 95% v IsoPropanol 5% v Trifluoro acetic acid 0.2% v



Analytical and overloaded injections on preparative column

It appears 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: takes time to prepare a new column and requires the use of more important amount of stationary phase.
- Use a classical recycling method, or perform “peak shaving”: will increase cycle time and therefore will increase the production time.

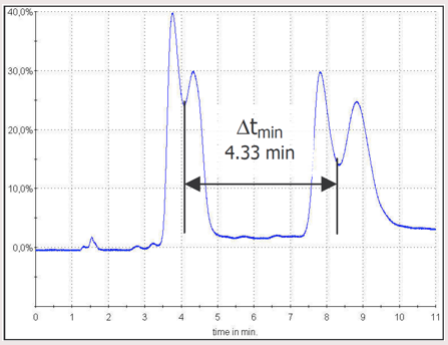
All these solutions imply a loss of both development time and production. .../

CASE STUDY 1 EXAMPLE WITH CHIRAL SEPARATION

A SIMPLE EXPERIMENTAL METHOD ALLOWS TO DETERMINE CYCLOJET PROCESS CONDITIONS ESPECIALLY THE CYCLE TIME AND THE INJECTED QUANTITY. THE METHOD IS DESCRIBED HEREUNDER:

- 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 much acceptable (see next figure).
- Measure the time between the minima of the initial injection and the recycled injection (as shown in next figure).

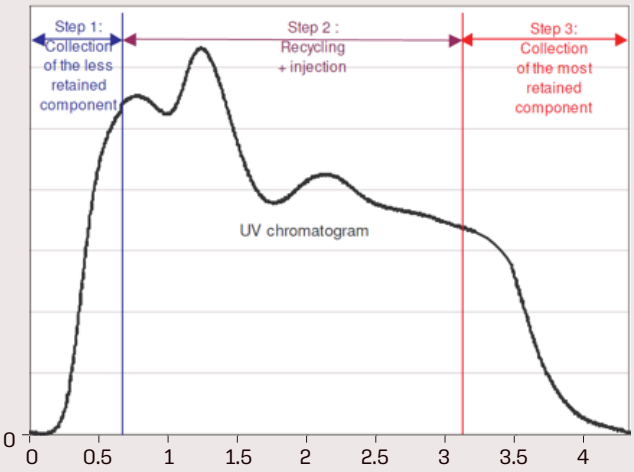
The cycle time for the Cyclojet operation will be between 0.9 and 1 x Δt_{min}.



Raw estimation of injected volume and cycle time

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

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}



Cyclojet UV Profile

IN CONCLUSION,

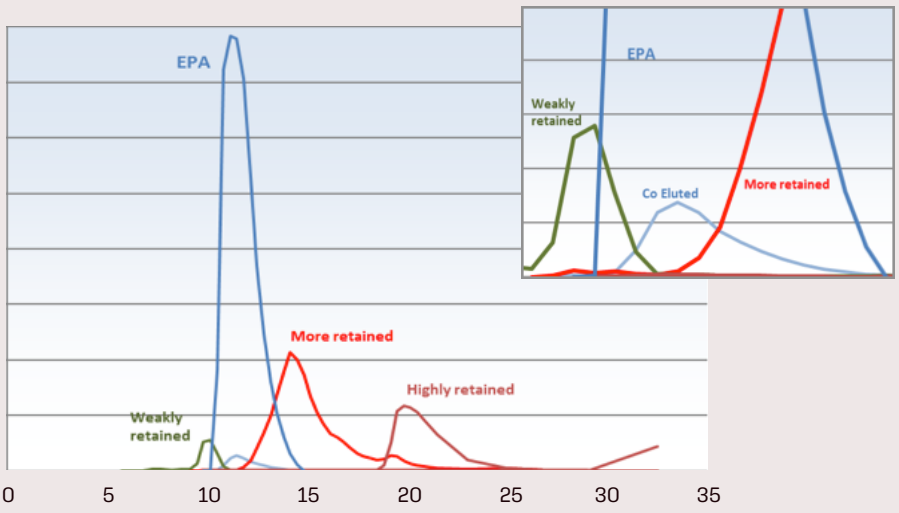
Cyclojet is a useful process for low resolution chromatographic separations. Applied to an unfavorable chiral resolution, it allowed to reach good purities and yields in short development time. Thus a significant gain in term of productivities and laboratory costs is highlighted.

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 on the following graphs.



Peak reconstruction profiles for feed fish oil

Data:

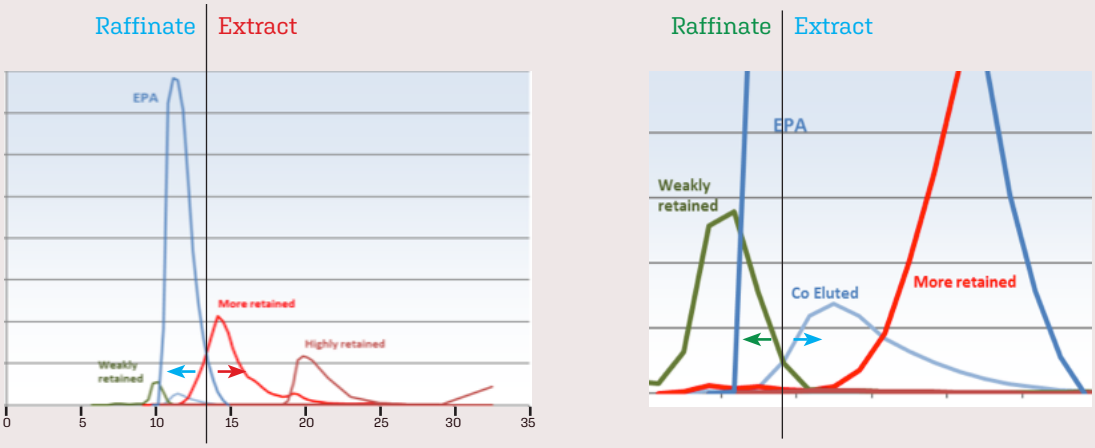
- Weakly retained impurities (WRI) are eluting close to EPA. Their purification is extremely difficult using a batch process and so they are to be removed by means of optimized process.
- Icosapent ethyl: Target product.
- More retained impurities are also reported their purification to be extremely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

.../

/...

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

Multi compounds products and low-resolution separation are key parameters to identify good candidate for 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.
A highly productive preliminary treatment can be imagined removing highly retained impurities.



Cut illustration of the two Cyclojet process steps

The above figures illustrate 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.

.../

/...

The performance results obtained on the purification of EPA contained in fish oil and by using the concept of two steps Cyclojet process are summarized in the following table.

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)

THE PRESENT CASE STUDY SHOWS

that for some difficult separations, as example chiral separation or peptides using chromatography technique, the Cyclojet process and more particularly the concept of two steps Cyclojet allow to increase productivity, purity and yield in order to make a process beneficial for the pharmaceutical companies.

NOTES

Services and technologies
for the life science industries

www.novasep.com

Contact our experts:
Hipersep.pilot@novasep.com

EUROPE
Novasep Equipment Solutions
Site Eiffel
81, boulevard de la Moselle - BP 50
54340 Pompey - FRANCE
Phone +33 383 497 000

AMERICAS
Novasep LLC
23 Creek Circle
Boothwyn, PA
19061 - USA
Phone +1 484 361 6000

ASIA
Novasep Asia
Building No.4,
Lane 1690, Zhangheng Road,
Zhangjiang Hi-Tech Park,
Shanghai 201203 - CHINA
Phone: +86 (0)21 6045 1600

INDIA
Novasep India
Four Square Elegant,
Block A, Flat 110,
Madhavapuri Hills, Road 5,
Hyderabad 50050 - INDIA
Phone: +91 9866573377

Ver. F | February 2022

How Hipersep® Pilot HPLC system / Design & layout: Bailly Créations