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Application Note

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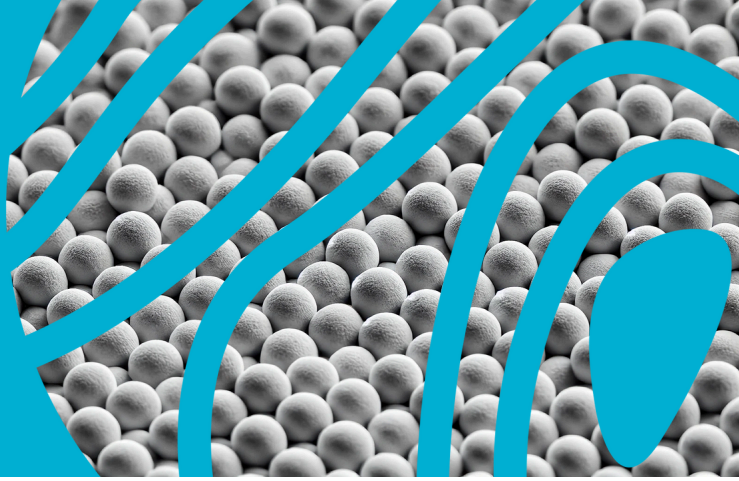

Introduction to Multi-Frequency Chromatography

Key words

HPLC, Preparative chromatography, Complex mixture purification, Intensified processes

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Abstract

Intensified HPLC processes often require specific hardware and multiple columns, making their implementation expensive and the process development complex. Multifrequency chromatography is a straightforward, intensified process that can be implemented on a single column managed by a standard HPLC system. This innovative downstream process is designed for the purification of complex mixtures, from lab to industrial scale, and its excellent performance leads to OPEX and CAPEX optimizations

Cromaoak's patented process, is a two-steps process conducted on one column. During the first step, a mixture to be purified is injected at a frequency F1, in parallel, collections are performed at the same time interval. An intermediate product is obtained and is then re-injected during a second step, at a second frequency F2, a non-integer multiple of F1. Through a strategic combination of two injection frequencies, the product is collected pure after the second step.

The present application note describes the general principles of multi-frequency chromatography, and illustrates its advantages compared with a traditional batch process.

Multi-Frequency Chromatography

Introduction

Multi-frequency chromatography is a technology particularly well-suited for the purification of complex mixtures, especially when the target molecule is surrounded by impurities with similar structures and properties.

This process is designed to perform repetitive injections at high frequency, with only a short time intervals between each injection.

Compared to batch and stacking modes, multi-frequency process maximizes the utilization of the stationary phase by drastically shortening the delay between injections (*Figure 1*).

- **Batch, traditional process:** A new injection is performed only after all molecules from the previous injection have been completely eluted (A).
- **Stacking, first step toward intensification:** Injections are scheduled to reduce the waiting time between the elution of the more retained molecules and the less retained molecules from the next injection (B).
- **Multi-frequency, intensified process:** Injections are scheduled with even shorter time intervales, maximizing column occupation and offering numerous advantages (C)(D).

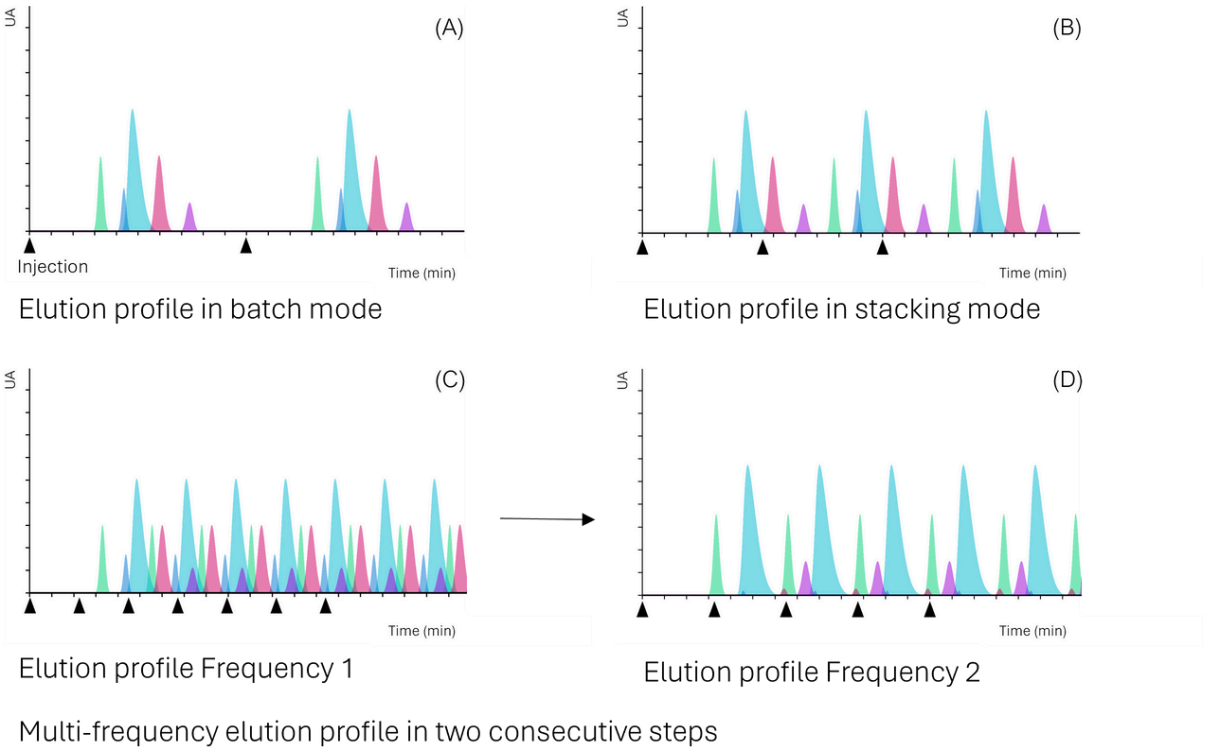

Figure 1: Illustration of elution profiles for the same separation Batch mode (A), Stacking mode (B) and Multi-frequency mode (C)(D).

Process Sequence

This process, patented by Cromaoak, is referred to as multi-frequency chromatography, as it involves two distinct injection frequencies named frequency 1 and frequency 2, which are applied during two consecutive steps.

The mixture to be purified is injected at the first time interval, in this example, 9 minutes. In parallel, but asynchronously, collections are performed every 9 minutes. An intermediate product is collected following this first step, its composition has evolved but does not yet meet the expected purity specifications (*Figure 2*).

The intermediate product is then injected at a second frequency, which is a non-integer multiple of the first frequency, in this example, 13 minutes. Similarly, collections are performed every 13 minutes (*Figure 3*).

Through a strategic combination of the two frequencies, the elution profiles shift between frequency 1 and frequency 2. Molecules co-eluted with the molecule of interest at frequency 1 are separated at frequency 2 by modifying the injection interval, specifically chosen as a non-integer multiple of F1.

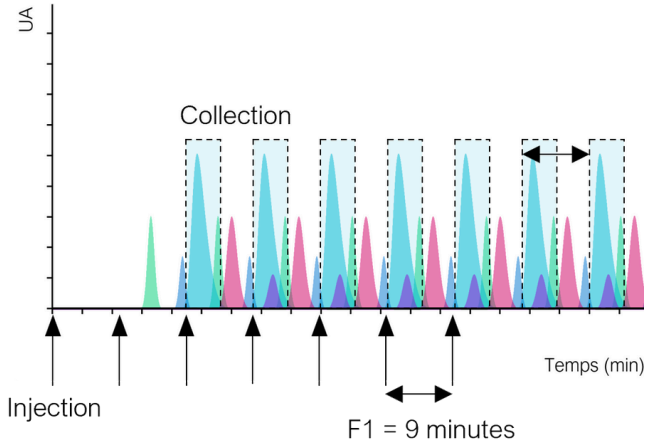

Figure 2 : Illustration of the first step of multi-frequency process: Frequency 1.

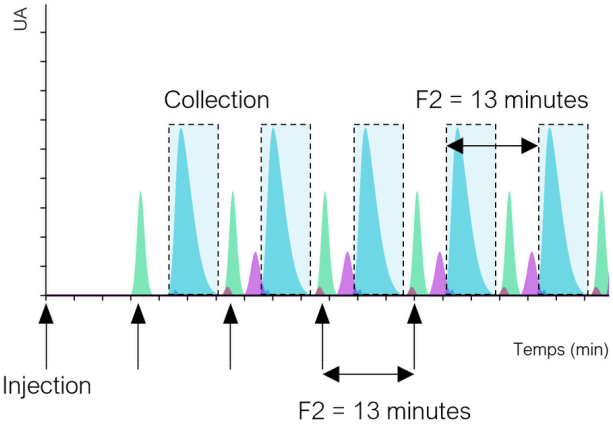

Figure 3 : Illustration of the second step of multi-frequency process: Frequency 2.

A video explaining our technology is available: [Cromaoak's Process]

Case Study Produced by Simulation

This case study, generated through simulation, aims to illustrate improvements typically achieved with a multi-frequency process compared to batch or stacking processes. The three scenarios are evaluated with the same mobile phase and stationary phase, with a 25 cm x 4.6 mm column. The mixture to be purified contains a target molecule at 37% concentration (*Figure 4*).

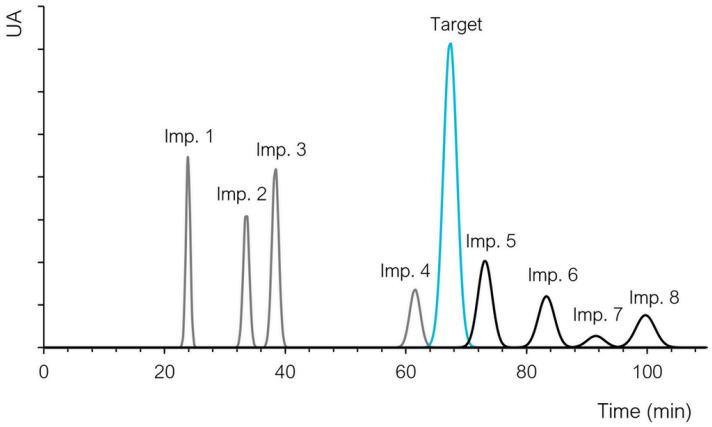

Figure 4: Mixture to be purified, injection of 10 µL.

Batch

Method

Elution is performed isocratically at a flow rate of 1 mL/min, using a mobile phase composed of 35% modifier. An injection of 850 µL is scheduled at t = 0 minutes, with collection occurring between 63 and 71 minutes.

Results

- Purity: 95.4 %
- Yield: 82.8 %
- Productivity: 0.336 kg purified/kg PS/Day
- Solvent consumption: 1 557 L/kg purified

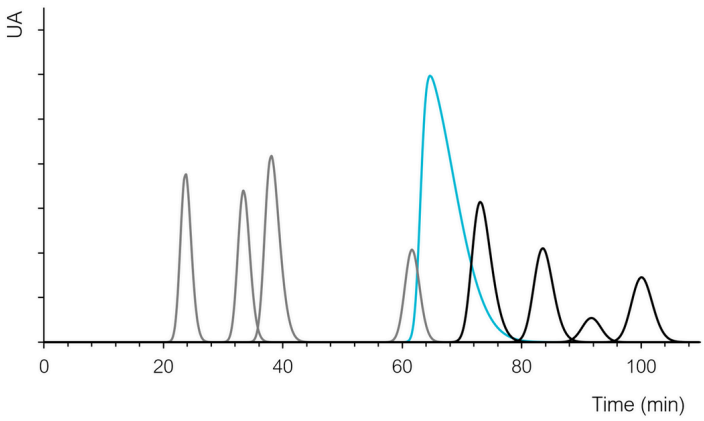

Figure 5: Batch chomatogram

Stacking

Method

Elution is performed isocratically at a flow rate of 1 mL/min, using a mobile phase composed of 35% modifier. An injection of 850 µL is scheduled every 85 minutes, with collection occurring between 63 and 71 minutes, after each injection.

Results

- Purity: 95.8 %
- Yield: 82.8 %
- Productivity: 0.432 kg purified/kg PS/Day
- Solvent consumption: 1 215 L/kg purified

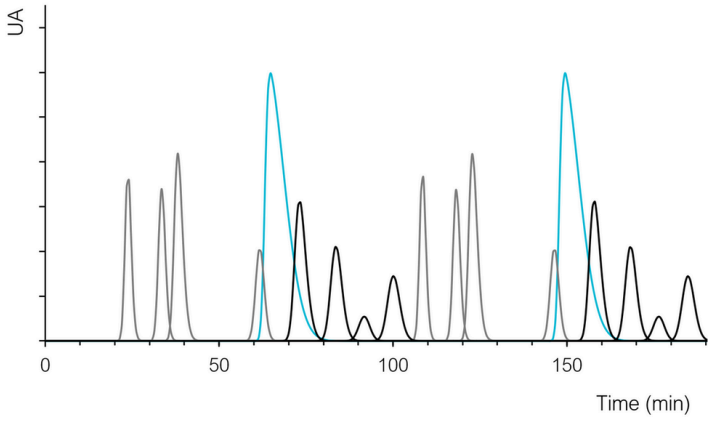

Figure 6: Stacking chromatogram

Multi-Frequency

Method

Elution is performed isocratically at a flow rate of 1 mL/min, using a mobile phase composed of 34% modifier for F1 and 40% for F2. In the first step, injections are scheduled every 17 minutes, with collections occurring between 84 and 94 minutes after each injection. In the second step, injections are scheduled every 13 minutes, with collections occurring between 18 and 25 minutes after each injection.

Results

- Purity: 95.9 %
- Yield: 92.0 %
- Productivity: 0.730 kg purified/kg PS/Day
- Solvent consumption: 714 L/kg purified

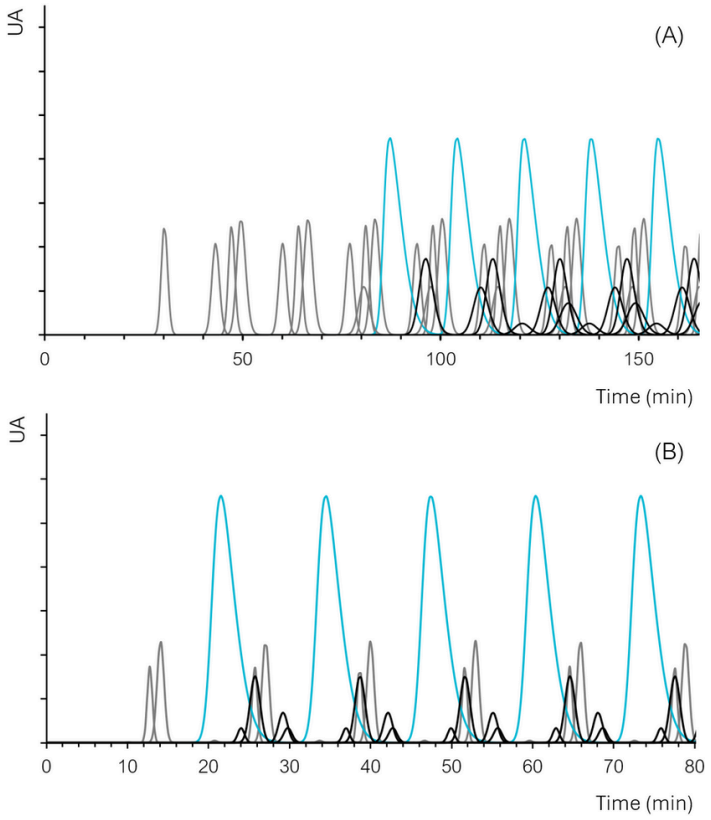

Figure 7: Multi-frequency chromatograms: Frequency 1 (A) et Frequency 2 (B).

Comparison

In this example, Multifrequency chromatography achiveves a 10% increase in yield, along with a twofold improvement in productivity and solvent consumption, while ensuring equivalent purity.

	Batch	Stacking	Multi-Frequency
Purity (%)	95.4	95.8	95.9
Yield (%)	82.8	82.8	92.0
Productivity (kg purified/kg PS/Day)	0.336	0.432	0.730
Solvent consumption (L/kg purified)	1 557	1 215	714
Estimated PMI (kg consumables/kg purif.)	1 402	1 094	643

Key Advantages of Multi-Frequency

Multifrequency chromatography maximizes column utilization, delivering optimized performance that can be quantified in various ways depending on the specific objectives.

Solvent Consumption

The amount of solvent required per kilogram of product purified can be reduced by a factor of 2 to 5, compared with a batch process.

- **Environmental footprint:** Enable a significant reduction in PMI (Process Mass Intensity) values, a metric used to assess the ecological impact of industrial processes.

- **OPEX:** Lower operating expenses due to reduced costs for solvent purchase, waste management, and energy required for reprocessing operations.

Productivity

An increase by a factor of 2 to 5 in the quantity of product purified per mass unit of stationary phase and unit of time (kg purified/kg PS/Day), compared with a batch process.

- **Production rate:** Possibility of increasing the annual production volume of an existing line by transitioning from a batch process to a multi-frequency process, using the same column size.

- **CAPEX:** When designing a new process, multi-frequency enables investment in smaller equipment while achieving the same annual production volumes as a batch process. This also reduces floor space requirements in production facilities.

Difficult separation

Multi-frequency chromatography enables the isolation of molecules and purification of mixtures extremely challenging to process using traditional processes.

- **OPEX and CAPEX:** Multifrequency achieves performance levels that make industrial-scale HPLC economically viable, even in cases where the high costs of a batch process would otherwise have declared it impractical.

- **Streamlined process:** A reduced number of purification and reprocessing steps, along with a simplified fractionation strategy, enhances operational efficiency.

Simplicity

Multifrequency chromatography is a straightforward, intensified process that can be implemented on a column managed by a standard HPLC system.

- **Single column process**: Unlike most intensified processes, multi-frequency can be run on a single column.

- **Simple sequence**: The events constituting a multi-frequency process are basic and closely resemble those of a batch process, involving an elution pump, injections, and collections at regular intervals.

Multifrequency chromatography is a powerful, durable and simple solution for optimizing existing purification process and developing new ones.

Applications

Chromatography is widely used in polishing steps to achieve specifications that cannot be attained employing other technologies such as filtration and crystallization.

Multi-frequency chromatography offers enhanced performance and is particularly valuable in the following fields of application:

Pharmaceuticals

- Oligonucleotides
- Peptides & proteins
- Vaccine component

Cosmetics & Perfums

- Plant extracts
- Peptides & proteins

Food & Nutraceuticals

- Fatty acids
- Polysaccharides
- Natural extracts

Multifrequency is well suited to most chromatography modes:

HPLC

- Reverse phase
- Normal phase
- Ion exchange
- Steric exclusion

LPLC

- Ion exchange
- Steric exclusion

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