

ADDED VALUE OF APPLYING CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC) TO BIOPROCESSING DOWNSTREAM PURIFICATION



APPLICATION NOTE AN1041

CPC APPLICATION BENEFITS

- Process an input sample containing 20% of the target compound to reach 95% purity and high recovery in a single step
- Devise a simpler process resulting in both the removal of a costly step and the availability of a direct, easy scale up to multiple tons production
- Avoid loss of target molecule and generation of waste usually associated with silica-based chromatography

ADDRESSED ISSUES

- Purification from a highly complex sample, derived from a nutritive fermentation broth mixture
- Prep HPLC chromatography, as initially considered, required additional, expensive steps with high solvent consumption
- Prep HPLC seemed unable to deliver purity at a level higher than 65% while target was 95%

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INTRODUCTION

Fermentation allows for the conversion of organic materials into relatively simple substances by using micro-organisms such as bacteria, Streptomyces, filamentous fungi, or yeast.¹

This process is commonly used both at R&D and production levels, especially with compounds which could not be obtained through either traditional synthetic organic chemistry, or from plant sources.² Fermentation has also proved to be an efficient way to dramatically reduce the use of solvents and avoid disruptions resulting from variations with plant source quality (e.g. seasonality).

As with other processes, each step can be expensive, hence a strong incentive to extract and purify the active compound from the nutritive solution in the most direct way.

In 2019, a leading, global chemical company requested Gilson Purification's expertise with centrifugal partition chromatography (CPC), to seek

ways to improve all key dimensions of isolating a specific compound from a complex fermentation broth: reduce the costs and environmental impacts (use of solvents and silica) and improve purity beyond the 65% threshold achieved through prep HPLC chromatography.



MATERIALS AND METHODS

Systems and solvents: Gilson has developed a unique portfolio of CPC solutions with systems optimized for work at lab, but also pilot and process scales. Several of these were used to address our client's needs.

A Gilson VERITY® CPC Lab system, including a CPC 250, connected to a PLC 2050 Purification System (Compact LC system) configured with a 50 mL/min quaternary gradient pump, UV/VIS detector, fraction collector, and Gilson Glider CPC control software were used for the feasibility study. (Figure 1)

A Gilson VERITY® CPC Pilot system, including a CPC 1000 PRO, connected to a PLC 2500 Purification System (Compact LC system) configured with a 500 mL/min quaternary gradient pump, UV/VIS detector, fraction collector, and Gilson Glider CPC control software were used for the scale up study.

Analytical HPLC was performed on a Hitachi LaChrom Elite® HPLC System configured with a photodiode array detector (PDA) (200–800 nm).

All organic solvents were of analytical or high-performance liquid chromatography (HPLC) reagent grade.

Sample: Crude dry extract from fermentation broth with an estimate of 20% (w/w) of target compound. The latter is identified with a retention time (Rt) at 5.28 min on the HPLC chromatogram of the crude sample analysis (Figure 2).

CPC Method:

Feasibility study on VERITY CPC Lab system

800 mg of crude dried extract diluted in 5 ml of the lower phase of the solvent system was injected into the CPC 250 in descending mode. The elution flow rate was 10 mL/min, extrusion flow rate 30 mL/min, and rotation speed 2000 rpm, with extrusion starting after 40 minutes of elution. Detection was fixed at 220 nm and scan at 200-400 nm.

Scale up study on VERITY® CPC Pilot system

10.5 g of crude dried extract diluted in 60 ml of the lower phase of the solvent system was injected into the CPC 1000 PRO in descending mode. The elution flow rate was 150 mL/min, extrusion flow rate 300 mL/min for a total runtime of 17 mn. Rotation speed was set at 1400 rpm and detection at 220 nm, scan at 200-400 nm. Process was run at room temperature.



Figure 1
Gilson VERITY® CPC Lab System

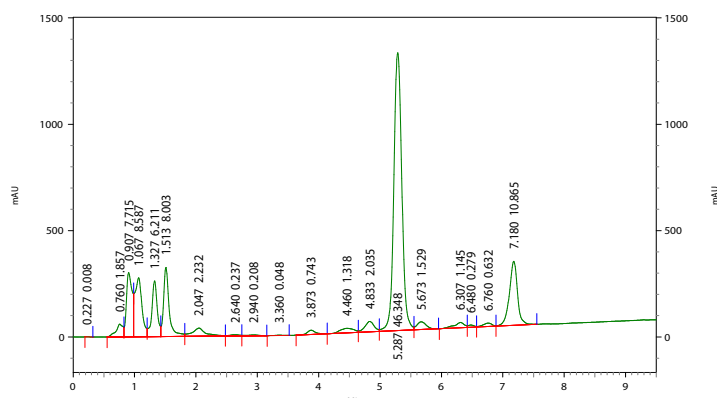


Figure 2
HPLC analysis of the crude sample at 220 nm

RESULTS AND DISCUSSION

Feasibility studies

The CPC separation of the crude sample resulted in a single-step global fractionation of the fermentation broth sample into three distinct groups (Figure 3).

After drying and performing HPLC analysis at 220 nm (Figure 4), the target compound is collected from Group 2 – 188 mg or 23.5% of the injected mass (cf. table 1) – with 94.2% purity of the target compound achieved (cf. figures 2, 3 and table 1).

At this stage, solvent consumption was already 55% less than with prep HPLC.

Scale up

A scale up of the method was performed using a CPC 1000 PRO resulting in the injection of up to 10.5 g per run. With a duration of 17 min per run, it proved possible to process up to 294 g of crude sample per 8 hours (Table 2).

Production was subsequently assessed for a fully automated, process scale CPC system, the VERITY® CPC Process (5 L). This demonstrated that up to 2 kg of crude sample could be processed per 8 hours with a recovery rate of 81% and achieved purity still at 94% (Table 2).

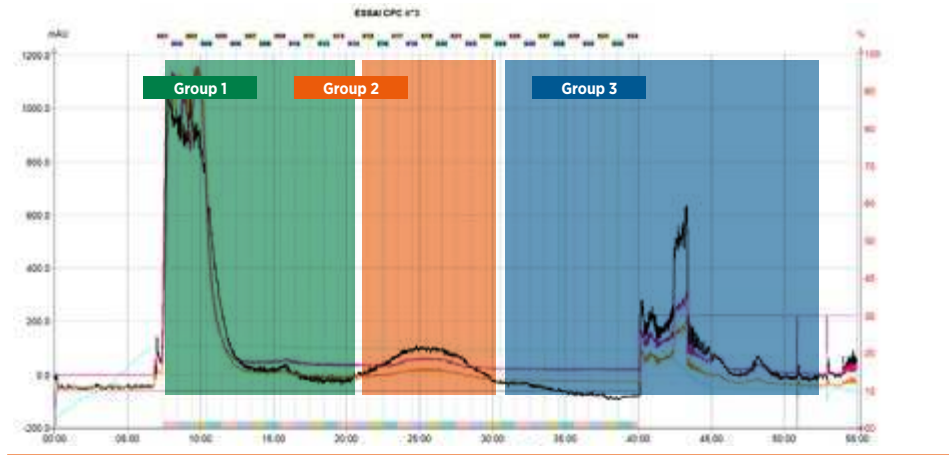


Figure 3

CPC chromatogram for an 800 mg injection of a sample with 20% target compound (254 nm, 220 nm, 263 nm, 280 nm and scan 200-400 nm)

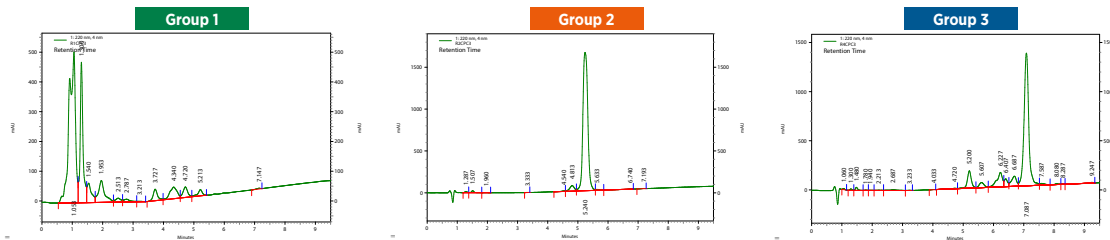


Figure 4

HPLC analysis 220 nm of groups 1, 2 and 3 from CPC run

Table 1

Mass balance / sample recovery and purity from a CPC run of 800 mg of dry extract

GROUPS	1	2	3	TOTAL
Fractions	1 – 13	14 – 23	24 – END	-
Fraction weight (mg)	391	188	102	681
Mass yield (% of 800 mg, original dry extract)	48.8%	23.5%	12.75%	85%
Target purity (HPLC 220 nm)	-	94,2%	-	

Table 2

Scale up on a VERITY® CPC Pilot system and estimated productivity on a VERITY® CPC Process system

	VERITY CPC PILOT 1000 PRO	VERITY CPC PROCESS 5 L
Injected sample (g/run)	10.5 g	Up to 73 g
Process output (g/run)	1.7 g	Up to 11.9 g
Injected sample (g/8hrs)	294 g	Up to 2044 g
Process output (g/8hrs)	47.6 g	Up to 333 g
Estimated target compound recovery	81%	

CONCLUSIONS AND BENEFITS

This study demonstrated the added value of CPC at achieving, in one step, an efficient separation from a fermentation broth. While the original prep chromatography process delivered the target compound with 65% purity, CPC allowed to achieve purity levels higher than 94% from a crude sample at 20%. Finally, the application of CPC already entailed a 55% reduction in solvent consumption at Lab level.

The complementary scale up study performed for Gilson VERITY Pilot and VERITY Process Systems showed the capability to achieve similar results while processing multiple tons per year in a GMP environment.

REFERENCES

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2. <https://www.pharmtech.com/view/fermentation-finds-fans-in-small-molecule-api-synthesis>

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