Scale up in Countercurrent Chromatography (CCC)

Loïc CHABANNY

Introduction

The Countercurrent Chromatography(ccc) is a preparative technique which allows the purification of compounds, from the mg to the kg scale. This technique uses a stationary phase and a mobile phase, both of them are liquid, but not miscible (biphasic system). The stationary phase is maintained in the column thanks to the application of a centrifugal rotary field, while the mobile phase is pushed through using a pump. The principle of a scale-up is to transpose conditions of separation that have been rapidly developed on a small machine towards one of more important volume. In order to get the same resolution it is necessary to keep the same amount of stationary phase. This parameter called Sf is influenced in CCC by the rotation speed and the mobile phase flow rate.
In this work, the separation of benzyl alcohol and para-cresol is achieved using Heptane / Ethyl acetate / Methanol / Water (1,4;0,1;0,5;1,0; v/v/v/v) as a solvent system, the stationary phase being the heptane-rich upper phase. Separation was first optimized in a 36ml rotor before being transposed to a 232ml rotor.

Results and discussion

After optimization, it was possible to maintain Sf=60% of stationary phase in the small rotor (8mL/min and 1900 rotations per minute (RPM)). The maximum amount of solute to be separated in these conditions is 160mg for the benzyl alcohol and 44mg for the cresol. Pressure reached 63bars when the equipment can support a maximum of 70 to 75bars. When working with the large rotor, two conditions were found to meet a Sf=60% (Table 1). A condition called soft condition (750rpm; 20mL/min), where pressure is low (26bars), and a harsher condition (1250RPM; 38mL/min), where pressure is high (63bars). The injected amounts of compound are higher, since the volume of the column is 6.5 times larger. However, the hourly yield is different whether soft or harsh conditions are used for benzyl alcohol for example, 1.2 g/h on the small rotor against 5.3 g/h in soft conditions and 15.4 g/h in harsh conditions. The choice is critical: low pressures to prevent leaks and breakdowns but with a lower yield, or greater pressures risking leaks, but providing better yields.

Moreover, it is possible to find optimal conditions on the large rotor where Sf reaches 73%. Yields are slightly lower than harsh conditions because the time of separation is longer (F = 14mL/min), that said, the injected amount per run is much greater (21% of the column), which has positive consequences on solvent consumption.

Table1 : *Overview of the conditions of scale-up*

Parameters	Small rotor (36mL)	Large rotor (232mL)		
	Descending	*Sweet conditions*	*Hard conditions*	*Optimal conditions*
F (mL/min)	8	20	38	14
RPM	1900	750	1250	1400
P (bar)	63	26	63	60
Sf (%)	60	62	60	73
V_{inj} (mL) *(%column)*	2 (6%)	22 (9%)	32 (14%)	48 (21%)
m_i BzOH inj (mg)	160	1 760	2 560	3 840
Yield benzyl alcohol (g/h)	1,2	5,3	15,4	9,6
m_i P-cresol inj (mg)	44	484	704	1 056
Yield P -crésol (g/h)	0,3	1,4	4,2	2,6
$V_{solvent\ run}$ expressed as Volume of column)	1,8	1,7	1,6	1,5
Vsolvent /g of purified benzyl alcohol	400	220	150	100

Conclusion

CCC is a technique little known but not without interests. This is an economical technique used in many fields (medicine, food, petrochemical...). Researches on the optimal conditions for purification of compounds are needed to give the best answers to the demands of industrial companies who are increasingly using this type of machine. This work on scale up reveals that the conditions to get efficient purifications can be multiple. This is why it will be down to industrial companies to set their criteria.

Université Claude Bernard Lyon 1

UCBL
Master Analyse et Contrôle
Bâtiment CPE
69622 Villeurbanne