

Purification of synthetic mixture in gradient mode by CPC/CCC

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Eight compounds from a test mixture were purified by Centrifugal Partition Chromatography (CPC). The diversity of the molecules of this mixture requires the use of gradient mode to reduce the time of separation and the consumption of solvent. CPC is accomplished with SCPC-250 from Armen instrument.

This device allows, through dedicated CPC software, to develop a method, including all the steps necessary for the separation in order to automate and simplify handling.



Keywords: Gradient, software, standard mixture, SCPC

Test mixture description

Structure	Reagent Chemical Name	Rt	Structure	Reagent Chemical Name	Rt
	Warfarin	0.81		Methyl 2-Acetamido-5-Bromobenzoate	2.18
	4-Bromobenzamide	1.35		Naphthalene	2.54
	Methyl 4-Amino-3-Methylbenzoate	1.51		Biphenyl	2.79
	Dipyridamole	2.02		Phenanthrene	2.96

Table 1: Structure of target compounds

The sample to be treated is a mixture of eight compounds [Table 1] covering a broad range of retention in reverse phase HPLC [Figure 1]. Compounds 1 and 2 are used to assess the recovery of purification process, 4 is a fluorescent yellow compound that can be visually followed, three others 6, 7 and 8 have close structures and allow to assess the performances of the system, two 3 and 5 are pH dependent.

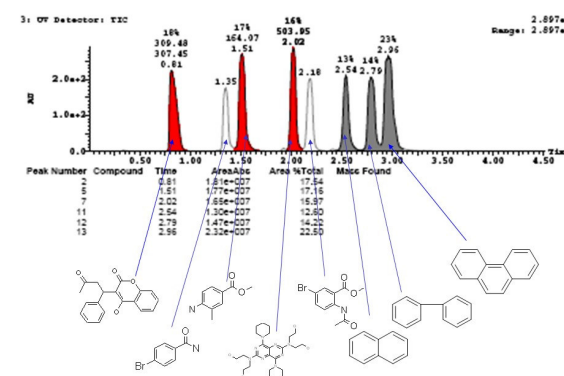


Figure 1: Analytical HPLC chromatogram of test mixture

Solvent system determination

System	hexane	acétate d'éthyle	methanol	eau
A	0	1	0	1
B	1	19	1	19
C	1	9	1	9
D	1	6	1	6
F	1	5	1	5
G	1	4	1	4
H	1	3	1	3
J	2	5	2	5
K	1	2	1	2
L	2	3	2	3
M	1	1	1	1
N	5	6	5	6
P	6	5	6	5
Q	3	2	3	2
R	2	1	2	1
S	5	2	5	2
T	3	1	3	1
U	4	1	4	1
V	5	1	5	1
W	6	1	6	1
X	9	1	9	1
Y	19	1	19	1
Z	1	0	1	0

Table 2: ARIZONA system.

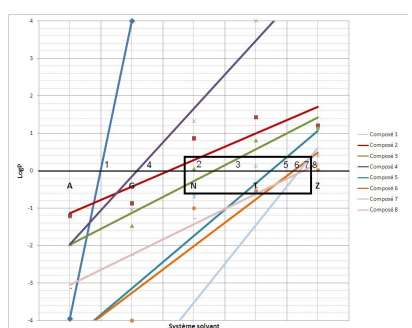


Figure 2: Presentation of the evolution of the coefficients of distribution of the 8 compounds in log10 according to systems.

The biphasic system chosen is hexane, ethyl acetate, methanol, water containing 0.2 % NH₄OH, or ARIZONA system [Table 2]. The coefficients of distribution of the eight molecules were measured in systems A, G, N, T and Z. Figure 2 gives the order of elution of compounds in CPC, to identify the range N-W as giving K_d close to 1 for the majority of compounds, and so to establish a gradient in descending mode (mobile lower phase) between systems N and W to clean all the eight molecules in a minimum of time.

SCPC runs and results

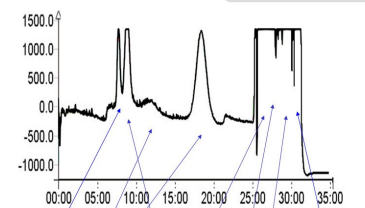


Figure 3: CPC chromatogram 100 mg.

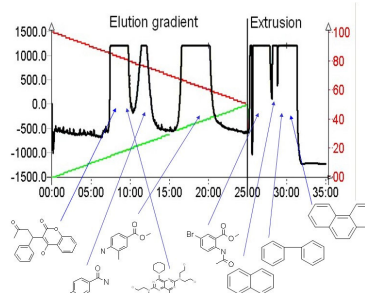


Figure 4: CPC chromatogram 200 mg

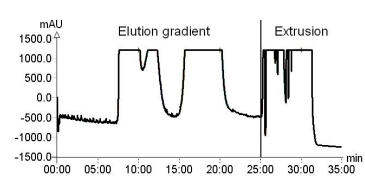


Figure 5: CPC chromatogram 300 mg loading

Conditions

Experiment with different loading of 100, 200 and 300 mg [Fig. 3, 4, 5] are carried on an Armen SCPC-250. Sample in DMSO, Mobile Phase A (Lower Phase of System N) - Mobile Phase B (Lower Phase of System W); gradient of elution: from 0 % to 50 % of Mobile Phase B in 25 min; extrusion with 100 % of C; detection 254 nm. All automatically pilot by Armen Glider Software : Stationary phase loading, equilibration, injection, gradient elution, extrusion [Fig. 6]

Commentary

The load experiment of 100 mg give fraction purity close to 100 % in both cases except for compound 8 in CPC which is of 75 % purity. The injection up to 300 mg gives comparable results. Beyond this loading, the stationary phase in CPC is destabilized this being probably due to the volume of DMSO used for injection to dissolve all the eight compounds

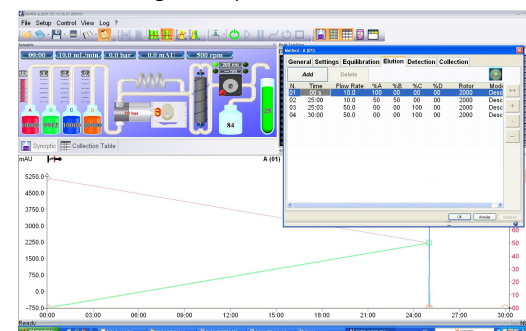


Figure 6: Armen Glider CPC software

Conclusion

This study allows identifying this technology as directly comparable in preparative HPLC for the purification of several compounds of very different polarities, without using expensive columns and with a gradient to optimise separations time and the consumption of solvent. It is also of note that by only changing the volume of the column, an inexpensive production capacity and a direct increase in scale [1].