

Heptane substitution by an agro-solvent in hydrostatic Countercurrent Chromatography.

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Introduction

CCC is a separation method which uses two liquids as stationary and mobile phases. For separation with this method heptane is typically used as solvent. This product comes from petrol industries and is a non-renewable product. Nowadays the preservation of planet earth is important so heptane tends to be replaced by agro-solvents such as limonene.

Limonene is a good candidate to replace heptane as solvent because their Poct/water are close (respectively 4,2 and 4,66).

Material

Experiments were made with hydrostatic instrument : a Shimadzu LC-6A liquid Chromatography pump was used, the CCC was a hydrostatic prototype of the ROUSSELET-ROBATEL company, the volume of the column was 33 ml. The detector used was a Shimadzu SPD-6A UV-spectrophotometric detector and the manometer is a TM manometer.

The stationary phase was heptane or limonene, the mobile phase was MeOH/water in different proportions. Separated products were methylparabene and diethylphtalate. As methylparabene is a dead time marker, Kd corresponds to Kd's diethylphtalate.

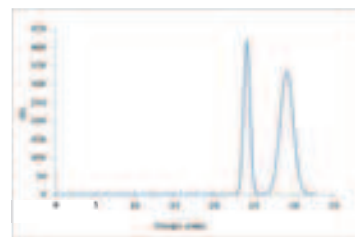
Results and discussion

Sf notion has to be introduced. Sf is expressed as a percentage and symbolizes the rate of stationary phase in the column. The first system solvent used was Heptane-MeOH/Eau (90/10) in condition 2500 rpm (rotation by minute) and a flow rate of 2 ml.min⁻¹. Then the system solvent was limonene/MeOH/Eau (90/10) in two conditions: the same as with heptane (2500rpm 2ml.min⁻¹) and in optimized conditions (3000 rpm 2 ml.min⁻¹). Results obtained were noted in the following table.

Conditions	Heptane		Limonene	
	Optimized	As with heptane	Optimized	
	2500 rpm 2ml.min ⁻¹	2500 rpm 2ml.min ⁻¹	3000 rpm 2ml.min ⁻¹	
dead time Tm (min)	5,05	7,84	6,99	
retention time diethylphtalate (min)	8,3	13,45	13,49	
Sf (%)	69,3%	52,4%	57,5%	
Pressure (bar)	65	15	17	
Kd	0,27	0,65	0,65	
efficiency of methylparaben	210	240	300	
efficiency of diethylphtalate N2	190	150	170	
Rs	1,72	1,84	2,44	

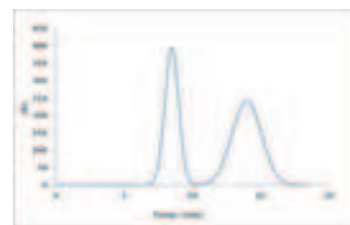
This table shows that with the same condition heptane is more retained than limonene by the column (Sf higher) but as Kd with limonene is higher than with heptane, the resolution with limonene is better. Chromatograms 2 and 3 clearly exhibit this result.

The solvent system with limonene in stationary phase can be used in higher rotation than with heptane in mobile phase because it generates a lower pressure. Values in the table chart show that the pressure in the same conditions for heptane and limonene were 65 and 15 bars, respectively. That is why the rotation can be increased to 3000 (the maximum for the instrument) for limonene, resulting in only 17 bar.

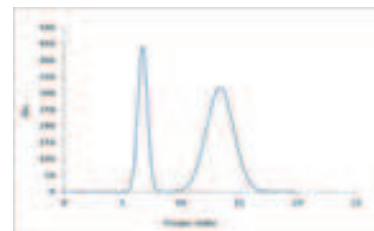


Chromatogram 1 : separation of the methylparabene diethylphtalate at 2500rpm 2 ml.min⁻¹ : Stationary phase heptane,

As the speed of rotation was faster the Sf was better so the resolution was better, chromatogram 2 and 3 show it precisely.



Chromatogram 2 : separation of the methylparabene diethylphtalate at 2500rpm 2ml.min⁻¹ : Stationary phase limonene



Chromatogram 3 : separation of the methylparabene diethylphtalate at 3000rpm 2ml.min⁻¹ : Stationary phase limonene

Conclusion

For a hydrostatic instrument, limonene provided a better separation than heptane at the same conditions. Furthermore as the pressure was lower with limonene (thanks to the weak interfacial tension between the two phases) conditions could be pushed to obtain a better resolution. To further optimize the separation, other parameters could be used such as the percentage of water in the mobile phase. Limonene will also be tested in a hydrodynamic instrument.

