Method Development and Preparative Purification of 2-Azabicyclo[2,2,1]hept-5-en-3one in Normal Phase and SFC Mode

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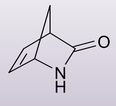
Introduction

The method development time for chiral separation can be significantly reduced by using parallel screening techniques. Two commonly used techniques to obtain pure enantiomers are HPLC and SFC. SFC utilizes liquid CO_2 which has a number of advantages over HPLC, usually shorter retention time, it allows higher flow rates, faster equilibrium and for preparative separation often results in smaller fraction volumes.

To identify the most efficient preparative method for the separation of 50 g of the racemic compound, 2-Azabicyclo[2,2,1]hept-5-en-3-on parallel screen in HPLC and SFC mode were performed. Both enantiomers were needed and with enantiomeric excess above 98%.

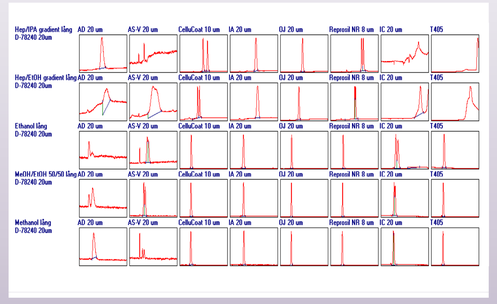


2-Azabicyclo[2,2,1]hept-5-en-3-on

Parallel Screen and Method Optimisation

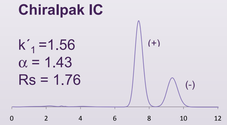
To scout for new separation methods we use Sepmatix 8x parallel systems in both SFC and HPLC mode. Using one pump, the patented flow control can regulate and deliver equal flow rates to eight different columns. The chromatographic data is visualized with the Sepmatix Screening Wizard software.

Parallel Sepmatix HPLC Screen and Optimisation



Screen conditions: 250 x 4.6 mm, 20 µm 1 ml/min,RT, gradient from 10 to 90 in 12 minutes

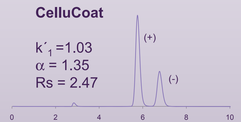
Chiralpak IC



k'_1 =1.56
α = 1.43
Rs = 1.76

Optimised conditions:
Heptane/Ethanol 50/50, 1 ml/min, RT

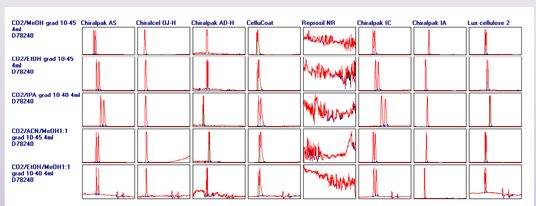
CelluCoat



k'_1 =1.03
α = 1.35
Rs = 2.47

Optimised conditions:
Heptane/Ethanol 85/15, 1 ml/min, RT

Parallel Sepmatix SFC Screen and Optimisation



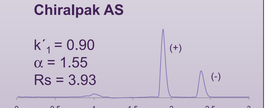
Screen conditions: 250 x 4.6 mm, 5 µm, 4 ml/min, 40°C, gradients from 5 to 40% modifier in 10 minutes

Chiralpak IC

k'_1 = 1.75
α = 1.39
Rs = 2.01

Optimised conditions: 20% Ethanol in CO_2, 4 ml/min, 120 bar, 40°C

Chiralpak AS



k'_1 = 0.90
α = 1.55
Rs = 3.93

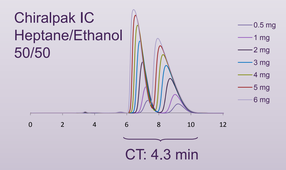
Optimised conditions: 20% Ethanol in CO_2 ,4 ml/min, 120 bar, 40°C

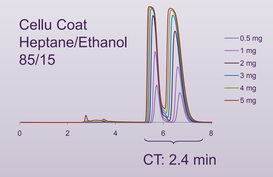
In both HPLC and SFC mode eight different columns and five different mobile phases were used in the screen and also the same set of stationary phases. A complete SFC screen for one sample through 8 columns and 5 solvents can be done in about 90 minutes and for HPLC it takes about 160 minutes.

Loadability on LC vs SFC

Loadability studies on analytical CSPs were performed in both modes to identify the throughput for the optimised conditions on the selected stationary phases. The injected amount was increased until touching bands and not further to get a fair comparison since fraction collection over a peak is more difficult in SFC.

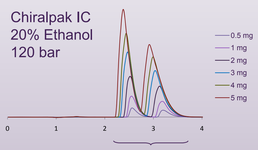
HPLC

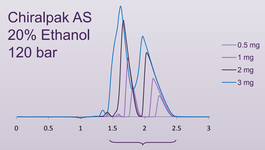




Sample dissolved in mobile phase resp Heptane/Ethanol 70/30, 100 mg/ml, RT, 20 µm.

SFC





Sample dissolved in mobile phase, 100 mg/ml, 40°C, 5 µm.

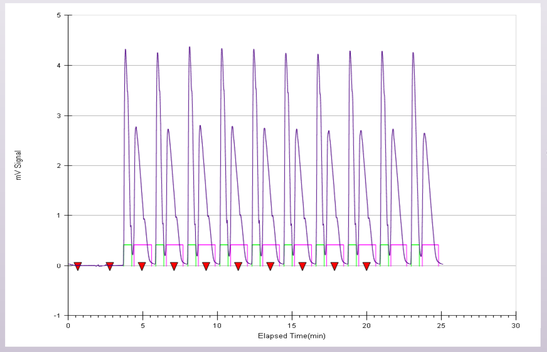
The SFC method on Chiralpak IC using 20% Ethanol in CO_2 proved to be the most efficient method for this compound even though the loadability was equal in HPLC mode on this CSP. Using the SFC method the solvent consumption is only 1/3 and the throughput almost 3 times higher.

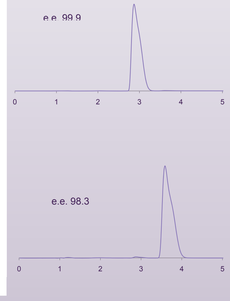
	LC		SFC	
CSP	CelluCoat	Chiralpak IC	Chiralpak IC	Chiralpak AS
Modifier	Heptane/Ethanol 85/15	Heptane/Ethanol 50/50	20% Ethanol, 120 bar	20% Ethanol, 120 bar
Loadability (mg)	3	4	4	2
Throughput (kg rac/kg/day)	0.7	0.5	1.4	1.1
Solvent consumption/g racemate	802 ml	1075 ml	300 ml	400 ml

Preparative Separation

Using the analytical loadability studies the injection amount for the preparative scale was calculated to be 170 mg/injection and this was also confirmed by preparative injections. In total, 50 g 2-Azabicyclo[2,2,1]hept-5-en-3-on was separated using stacked injections and the obtained yield and enantiomeric excess were as expected from the loadability studies.

Preparative Stacked Injections and Purity Analysis on Chiralpak IC





Preparative conditions: 30*250 mm, 20% Ethanol in CO_2, 270 mg/ml in Ethanol, 110 mg/ml, 120 bar, 40°C
Injection amount: 170 mg
Cycle time: 2.1 minutes

Analytical conditions:
20% EtOH in CO_2,
4 ml/min, 120 bar, 40°C

Even though the injected amount was only 170 mg the isomers were separated and isolated with required enantiomeric excess and yield (94%) within 11 hours and with a solvent consumption of only 14 liters of Ethanol.

Conclusion

- The time spent on method screening can be significantly reduced by the use of parallel screening in both HPLC and SFC. The column screening could be carried out eight times faster than conventional HPLC and SFC systems.
- Loadability of the compound at touching bands on Chiralpak IC was equal in HPLC and SFC mode, but higher throughput and lower solvent consumption makes the SFC method more efficient.
- A short cycle time, use of stacked injections, automatic fraction collection and low solvent consumption made it possible to use a 30*250 mm preparative column for the separation of 50 g 2-Azabicyclo[2,2,1]hept-5-en-3-on.

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