Chiral SFC Method Development of Immobilized Polysaccharide-Derived CSPs Using Non-Conventional Modifiers

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Abstract

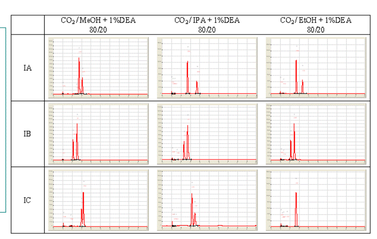
Chromatographic resolution of chiral molecules has been widely used in the pharmaceutical and biotech industries to support the early stages of drug discovery and development. For early stage clinical trials, small quantity of pure enantiomers can be obtained effectively and efficiently by using either HPLC or SFC. Recently, supercritical fluid chromatography (SFC) has gained increasing popularity due to its perceived "greener" operations. As with HPLC, for the separation of enantiomers, polysaccharide-derived chiral stationary phases (CSPs) prevail in SFC also. In this chromatographic mode, successful chiral method development is normally achieved using alcohols (or their combination) as eluting modifiers with very high success rates. However, due to the complexity of many molecules in development, issues are sometimes encountered with solubility, stability and/or racemization.

This poster describes the potential advantages of using solvents of medium polarity (different from alcohols) in SFC to enhance solubility and to control sample stability. Moreover, the inclusion of non-conventional solvents in the screening would supply alternative selectivity profiles, leading to even higher success rates. Method development strategies will be presented with those solvents and key parameters to be considered on 6 versatile polysaccharide-derived CSPs. Detailed experimental conditions and overall performance will be presented

SFC as an efficient screening tool for chiral molecules

PRIMARY SCREENING

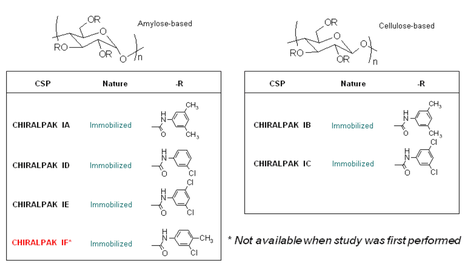
- Co-solvents: Alcohols and Acetonitrile
- Normally with high success rate!
- Broadly accepted, But……
- Stability issues
- Solubility issues
- More success rate for tough samples



Introduction

- CTI and Pfizer Pharmaceuticals in Groton started the project in 2011. A joint project was setup to learn more about the use of non-conventional modifiers for the following reasons:
  - ✓ Stability issues
  - ✓ Solubility reasons (prep applications)
  - ✓ Increase of screening success rate

Structure of CSPs used in the study



CSP	Nature	-R
CHIRALPAK IA	Immobilized	
CHIRALPAK ID	Immobilized	
CHIRALPAK IE	Immobilized	
CHIRALPAK IF*	Immobilized	

CSP	Nature	-R
CHIRALPAK IB	Immobilized	
CHIRALPAK IC	Immobilized	

** Not available when study was first performed*

SFC Conditions used in the study

System	Berger Analytical System
CSPs	IA , IB , IC , ID , IE
Column	250 x 4.6 mm, 5 µm
Mobile Phases	A: CO_2, B: Modifier (co-solvents) See next slide
Gradient	5 – 50% B gradient at 6.5%/min, hold for 3 mins, re-equilibrate for 2 mins
Temp.	40°C
Pressure	120 bar
Detection	DAD: 215, 230, 254 nm
Flow	4.0 mL/min
Injection	10 µL
Run Time	12 min

Non-Conventional Co-Solvent Systems

- Evaluations of ethyl acetate, THF and DCM
- Use of immobilized-type polysaccharide CSPs
- Parameters investigated:
  - ✓ Elution strength of those solvents in SFC
  - ✓ Role of the addition of alcohol in the elution and the success rate
  - ✓ Role of additives for the elution and resolution of acidic and basic molecules

Evolution of screening conditions in the study

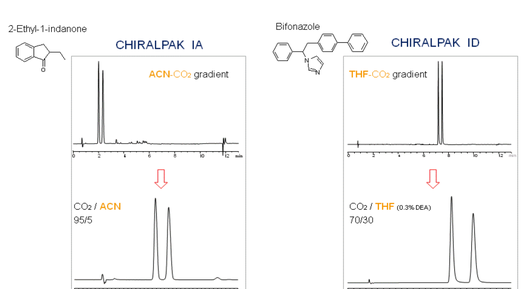
Initial setup
- 100% THF
- 100% EtOAc
- 50% DCM/50% MeOH

Revised setup
- 95% THF/5% MeOH
- 90% EtOAc/10% MeOH
- 90% DCM/10% MeOH

• 0.1% IPAmine added to co-solvents for basic molecules → • 0.2% IPAmine added to co-solvents for basic molecules

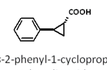
•No acid additive for acidic molecules → • 0.2% TFA added to co-solvents for acidic molecules

From gradient to isocratic

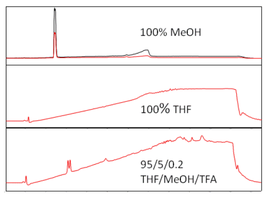


Bringing selectivity with non-conventional solvents

CHIRALPAK IA

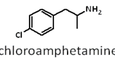


Trans-2-phenyl-1-cyclopropane carboxylic acid



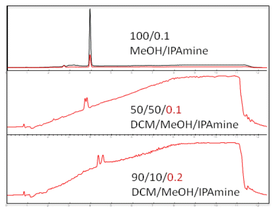
Bringing selectivity with non-conventional solvents

CHIRALPAK IC



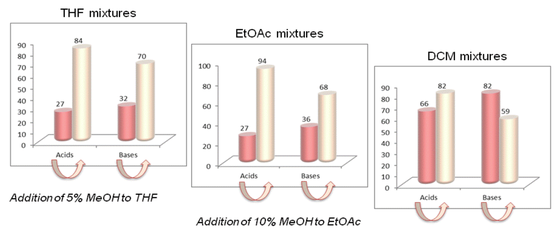
p-chloroamphetamine

Influence of % basic additive



The elution strength- effects of adding MeOH

Statistics on all runs and conditions tested (% elution)



Addition of 5% MeOH to THF

Addition of 10% MeOH to EtOAc

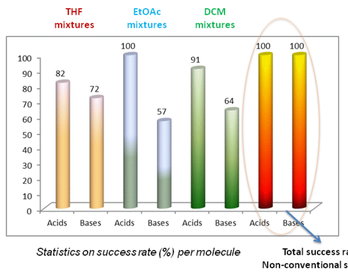
From 50% MeOH to 10% MeOH in DCM

Comparisons between 3 sets of conditions

	ACIDS	Alcohols	Old Ext. run	New Ext. run
acid 1	trans-2-Phenyl-1-cyclopropane carboxylic acid	8	4	10
acid 2	Bis-N-Cbz-DL-Lysine	6	none eluted	3
acid 3	Sulindac	6	2	4
acid 4	Suprofen	8	4	12
acid 5	Pycnogenole	11	4	12
acid 6	Dichloroprop	2	3	8
acid 7	Fenoprofen, Ca-Salt	0 resolved	3	5
acid 8	2(4-HO-PheO)-propionic acid	5	4	13
acid 9	3-Phenylbutyric acid	1	1	6
acid 10	2-Phenylbutyric acid	0 resolved	0 resolved	4
acid 11	2-Phenylpropionic acid	0 resolved	0 resolved	3
	BASES			
		Alcohols	Old Ext. run	New Ext. run
basic 1	Mebeverine HCl	9	4	6
basic 2	Propafenone HCl	9	5	7
basic 3	Homatropine	7	1	2
basic 4	Labetalol HCl	5	1	4
basic 5	Verapamil HCl	4	3	7
basic 6	Chlorpheniramine HCl	6	5	10
basic 7	Acebutolol	4	1	2
basic 8	Atropine	6	3	1 (IC)
basic 9	Ofloxacin	2	none eluted	1 (ID)
basic 10	Diperodon HCl	11	10	13
basic 11	Ketamin HCl	2	5	8
basic 12	Haloled	12	3	1 (IA)
basic 13	Salmbutamol HCl	4	0 resolved	1 (IA)
basic 14	DL-p-Chloroamphetamine HCl	0 resolved	3	7

Number of runs where there was resolution observed

The success rate per molecule



Statistics on success rate (%) per molecule

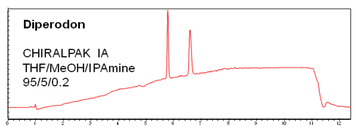
Total success rate Non-conventional solvents

- All molecules resolved in the extended solvent range
- For acidic compounds, EtOAc leads to higher success rate
- For bases, it may be important to further investigate higher % basic additive, although all of them were separated as well

Overview of conditions tested - THF

THF/MeOH 95/5 + 0.2 additive

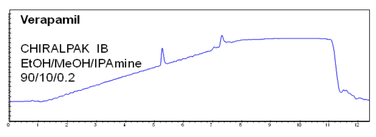
- Proper elution strength (>70%)
- Resolution success rate:
  - ✓ 82% for acids
  - ✓ 72% for bases



Overview of conditions tested - EtOAc

EtOAc/MeOH 90/10 + 0.2 additive

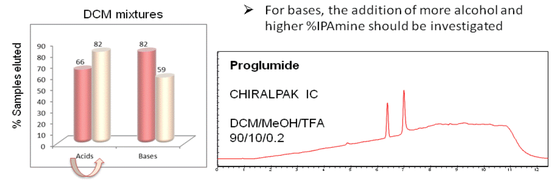
- Proper elution strength (>70%)
- Resolution success rate:
  - ✓ 100% for acids
  - ✓ 57% for bases
- For bases, the addition of more alcohol and higher % IPAmine should be investigated



Overview of conditions tested - DCM

DCM/MeOH/additive
Acid: 50/50/0 to 90/10/0.2
Base: 50/50/0.1 to 90/10/0.2

- Different tendencies for acids and bases
  - ✓ Acids elute better and reach higher success rates with 10% alcohol
  - ✓ Bases need more alcohol for elution and resolution
- Resolution success rate:
  - ✓ 91% for acids
  - ✓ 64% for bases
- For bases, the addition of more alcohol and higher %IPAmine should be investigated



Column Performance Comparisons

Total 25 compounds (acids and bases)					
Co-solvents	IA	IB	IC	ID	IE
95% THF/5%MeOH	10	6	14	12	7
90% EtOAc/10%MeOH	13	11	13	12	9
90% DCM/10%MeOH	11	7	9	8	8
Total Hits	34	24	36	32	24
Co-solvents					
95% THF/5%MeOH	IC > ID > IA > IE > IB				
90% EtOAc/10%MeOH	IC = IA > ID > IB > IE				
90% DCM/10%MeOH	IA > IC > ID = IE > IB				
Totals	IC > IA > ID > IE = IB				

Conclusions

- The use of extended solvent range in SFC for chiral applications offers advantages to:
  - ✓ Increase solubility of the samples
  - ✓ Avoid instability of molecules sensitive to alcohols
  - ✓ Broaden selectivity profiles and increase success rate
- Screening strategies should be adapted accordingly in terms of co-solvent composition and presence of additives
- Other non-conventional solvents (MtBE, etc) and other additive combinations could be further explored

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