A Comparative Study of Fast Orthogonal Chiral Screening Methods by SFC and Normal Phase HPLC

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Objective

•Develop fast and comprehensive chiral screening systems where chiral separation method development can be achieved in two to three hours using both SFC and normal phase HPLC under gradient mobile phase conditions

•Reduce optimization time needed to provide a transferable gradient or isocratic chiral method

•Increase productivity by reducing the method development time

•Reduce solvent consumption and waste disposal expenses further reducing the cost

SFC Chiral Screening System

Thar automated method development station
UV/Vis detector with wavelength monitored at 254 nm or 214 nm
Six column switching thermal control oven
Six solvent introduction gradient pump
Flow rate: up to 10ml/min

HPLC Chiral Screening System

Agilent HP1100 system with 8 columns switching capabilities
DAD detector with wavelength monitored at 254 nm and 214 nm
Chiralyzer detector available for additional chiral detection capability
Four channel solvent introduction gradient pump

SFC and HPLC Chiral Stationary Phases

"Fast screening" SFC chiral Columns from Chiral Technologies:

ChiralPak AD, 4.6x100mm, 5µm
ChiralPak AS, 4.6x100mm, 5µm
Chiralcel OD, 4.6x100mm, 5µm
Chiralcel OJ, 4.6x100mm, 5µm
ChiralPak IA, 4.6x100mm, 5µm (immobilized version of ChiralPak AD)
ChiralPak IB, 4.6x100mm, 5µm (immobilized version of Chiralcel OD)

Supelco Sigma-Aldrich Chirobiotic TAG 4.6x150mm, 5µm

Samples Selection and Prep

Fifty four commercially available chiral racemic compounds were studied. A similar number of chiral acidic, basic and neutral molecules including chiral amines, carboxylic acids, derivatized amino acids, and neutral molecules were included.

Samples were dissolved in 50/50 (v/v) methanol/ethanol at concentration of 1 mg/ml

HPLC Mobile Phase Conditions

Mobile phase:
Heptane with 2-70% modifier gradient in 5 minutes with post column equilibration of 2 minutes

Modifiers:
- Methanol/ethanol (50/50 v/v)
- 0.1% v/v Diethylamine (DEA) in methanol/ethanol (50/50 v/v)
- 0.1% v/v trifluoroacetic acid (TFA) in methanol/ethanol (50/50 v/v)
- DEA and TFA (0.1% v/v each) in methanol/ethanol (50/50 v/v)

Column pressure drop: ~100 bar
Flow rate: 2.5 mL/min
Temperature: 23 °C
Injection volume: 5µl
Detection: DAD at 214 and 254 nm

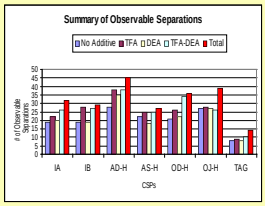
SFC Mobile Phase Conditions

Mobile phase:
Carbon dioxide with 2-70% modifier gradient in 4.6 minutes with post column equilibration of 1 minute

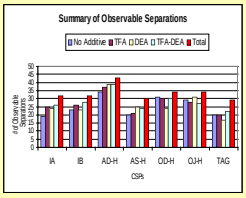
Modifiers:
- Methanol/ethanol (50/50 v/v)
- 0.1% v/v Diethylamine (DEA) in methanol/ethanol (50/50 v/v)
- 0.1% v/v trifluoroacetic acid (TFA) in methanol/ethanol (50/50 v/v)
- DEA and TFA (0.1% v/v each) in methanol/ethanol (50/50 v/v)

Back pressure control: 100 bar
Flow rate: 5.0 mL/min
Temperature: 32 °C
Injection volume: 5µl
Detection: UV at 214 or 254 nm

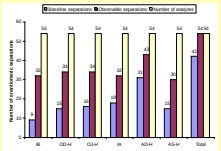
Summary of HPLC Chiral Screen Results



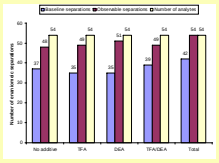
Summary of SFC Chiral Screen Results



Summary of SFC Results Based on Individual Columns

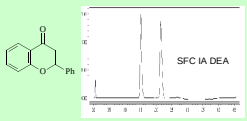


Summary of SFC Results Based Mobile Phase Modes

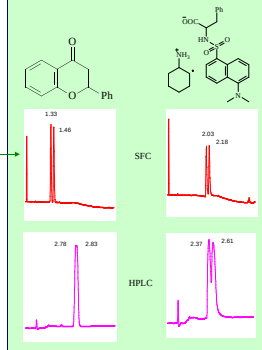


Comparison of HPLC vs. SFC

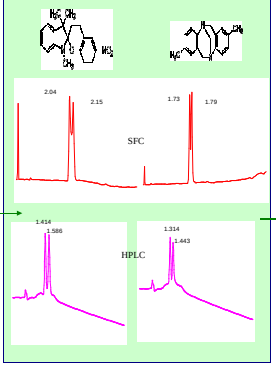
Comparable results were achieved on HPLC and SFC for this compound while SFC often offers slightly better efficiency



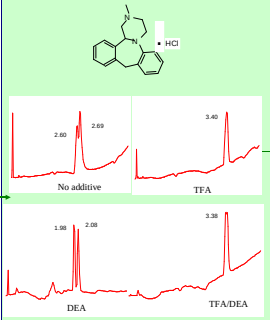
Comparison of HPLC vs. SFC Results On OJ-H Column



Comparison of HPLC and SFC Results on OD-H Column



Effects of Mobile Phase Additive for SFC on OD-H Column



Conclusions

A comprehensive chiral screen using SFC fast gradient methods can be done in around **two** hours for 24 methods.

A comprehensive chiral screen using HPLC fast gradient methods can be done in about **three** hours for 24 methods.

SFC and HPLC provide similar, but not identical results. They are both useful for chiral method screening.

SFC shows improved resolution over HPLC due to better efficiency for many cases

AD, OD, AS, and OJ columns provide separations for most compounds. IA and IB immobilized versions of AD and OD shows additional selectivities.

1st International Conference on SFC Sep 23-25, 2007 Pittsburgh, PA

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