Approaches Towards Implementation of Supercritical Fluid Chromatography-Mass Spectrometry (SFC-MS) for High-throughput Analysis of Pharmaceutical Compounds

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Abstract

High performance liquid chromatography (HPLC) is the most widely use separation technique within the pharmaceutical industry. Due to the growing need for high speed and high quality separations other techniques such as SFC are now being considered. A key advantage of SFC is minimal solvent waste, which is particularly important in preparative SFC, leading to fast sample recovery. Hence it is important to explore whether SFC, which also promises to be cheaper and more environmentally friendly than conventional HPLC, can be applied more widely as a complimentary method.

The objective is to develop a generic method focusing on speed by using smaller 50mm columns. Compounds with certain common substructures show peak splitting on the 2-ethylpyridine column. An approach to investigate the problem and possible solutions are presented.

Experimental approach

- Diverse training set (20 compounds)
- Small focused library of compounds with similarity in structures
- Direct comparison of SFC and HPLC
- Predictor- Map data retention against physico-chemical property

Peak splitting: artefact or reality ?

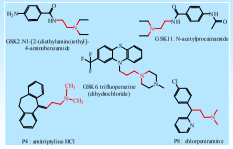


Figure 1: Structures of compounds showing peak splitting

- Isomers/ stereoisomer:- In source CID-MS, NMR
- Attempt to separate peaks (Figure 2)
- Peaks due to solvation:- MeOH / DMSO (Figure 3)
- Choice of solvents for
  - •Sample preparation (Figure 3)
  - •Modifier (Figure 3)

Analyse peaks separately

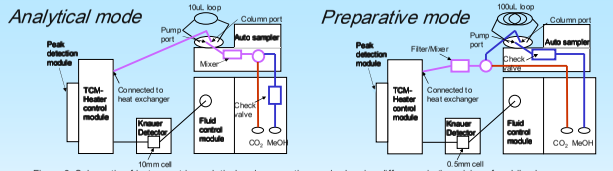
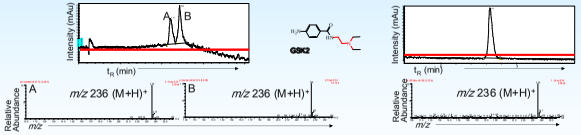


Figure 2: Schematic of instrument in analytical and preparative mode showing difference in the mixing of mobile phase



Difference in mixing of the mobile phase

- Analytical mode - CO2 and MeOH mixed before injection - Two peaks
- Preparative mode - CO2 and MeOH mixed after injection - One peak

Biphasic system or solvated molecule ?

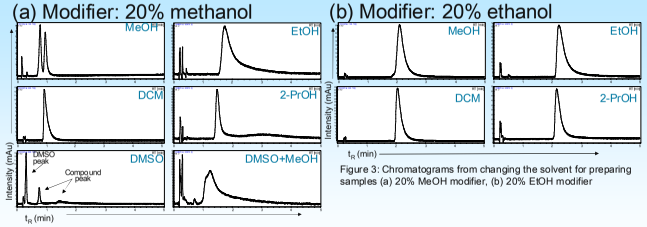


Figure 3: Chromatograms from changing the solvent for preparing samples (a) 20% MeOH modifier, (b) 20% EtOH modifier

- MeOH or DMSO may form solvated species
- DCM - single sharper peak shape

Possible solution: Ammonium acetate as additive

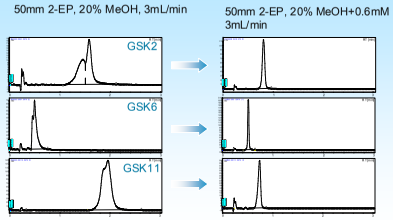


Figure 4: UV chromatograms : peak splitting on 2-EP and no splitting using additive ammonium acetate

Ammonium acetate additive for 2-EP columns
- ✓ Single peaks
- ✓ Sharp peaks
- ✓ Faster elution

Conclusions

- ✓ Peak splitting - Biphasic system or Methanol\DMSO solvated molecule
- ✓ DCM solvent - Improved peak shape for this class of compounds
- ✓ Ammonium acetate - Improved peak symmetry and separation.
  - Sharp single peak and early elution on 2-EP column
- ✓ SFC and HPLC - SFC gives greater coverage and is significantly faster compared to HPLC for this class of compounds

Acknowledgements

Improved peak symmetry and separation using ammonium acetate additive

250mm Cyano column,
25% MeOH with/without additive, Flow : 4mL/min

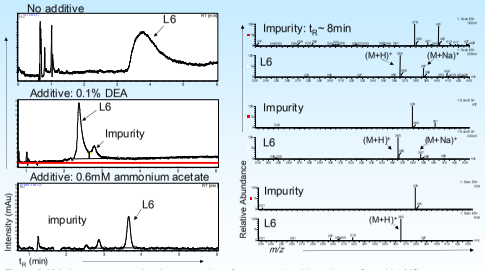
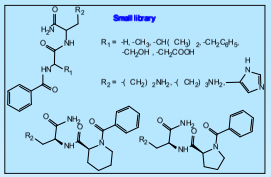
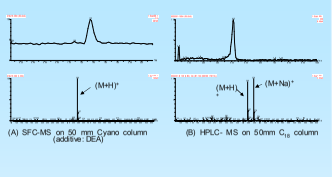


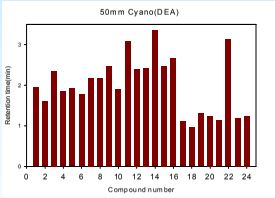
Figure 5: UV chromatograms showing separation of compound and impurity confirmed by MS

- Impurity co-eluting with DEA as additive
- Peak symmetry improved using ammonium acetate

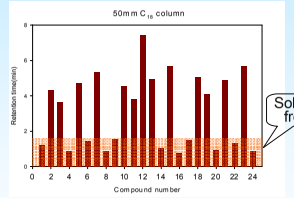
SFC and HPLC







Library analyzed with 50mm Cyano (SFC-MS)



Library analyzed with 50mm C_{18} (HPLC-MS)

- SFC gives greater coverage for the focussed library as the data suggests
- Nearly 50%(11 of 24) not compatible with generic HPLC method, eluting with the solvent front
- Retention related to the molecular structures: Compounds with open side chain elute later than those with ring structure

References:

1. Pinkston, J. D.; Stanton, D. T. Wen, D.; *Journal of Separation Science,* 2004, 27, 115-123.
2. Zheng, J.; Taylor, L. T.; Pinkston, J. D.; Mangels, M. L. *Journal of Chromatography A,* 2005, 1082, 220-229.