

A highly automated 5 pump, 4 detector super-critical fluid chromatography (SFC) system for chiral separation in drug discovery

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Why use chiral separations?

In drug discovery, many compounds are chiral. In some cases, individual enantiomers have unique and valuable biological properties.

In vitro and *in vivo* biological tests need to be performed using individual enantiomers, so that the stereo specific biological properties of drug candidates can be measured.

Often, chiral resolution is most efficiently achieved using column chromatography.

Reviews on the medical benefits of chirality in pharmaceuticals:

Agranat, H. Caner, J. Caldwell. *Int. Rev. Drug Discov.*, 1, 2002, 75.

H. Caner, E. Gonzalez, L. Levy, J. Agranat. *Drug Discov. Technol.*, 1(3), 2004, 105.

Why use super-critical fluid chromatography mass spectrometry (SFC/MS) system?

SFC can be used with modifier (normally MeOH) gradient to perform "generic" gradient analysis of wide range of compounds in a way that is generally complementary with RP-LC.

SFC is generally the best way to perform gradient elution for normal phase separations (sufficiently broad composition range).

Most of the best chiral columns work best in normal phase.

SFC faster and better loading than NP-LC (of which we do a lot).

SFC is MS compatible. This is a crucial efficiency component because it permits collection of **only** the particular desired peaks.

Cost of CO₂ operation is less than liquid solvents.

Fractions are conveniently (and efficiently) obtained in a minimal volume of modifier for easy recovery of purified compounds, with no water in fractions minimizing evaporation bottleneck and saving energy.

In our hands, chiral method development takes the least number of injections when using gradient SFC to screen column and mobile phase conditions.

Early efforts in preparative SFC/MS with open bed collection:

F. Wang, M. Barboi, T. Heath, D.S. Kassat, *Appl. Colloid Mass Spectrom.*, 11, 2001, 2007.

Z. Zhang, M.H. Thiele, G.E. Fatica, J.H. Blumens, *Org. Process Res. Dev.*, 6, 2002, 705.

Business objectives for our gradient SFC/MS based chiral purification platform

Routinely inject >100 mg and yield >10 g per day (1g/hr) of high purity (95-99% ee) for any number of chiral compounds (1-20) with >90% success rate, crucial for achieving throughput and fast turnaround.

Where desired, achiral purification of 100 compound parallel synthesis libraries (only when there is a need that can't be readily solved with LC/MS based purification).

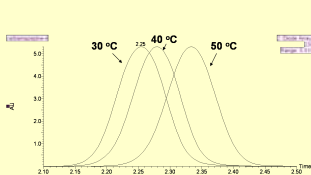
Flow > 100g/min.

Open bed collection under atmospheric conditions to simplify process and enhance safety (**must be safe and efficient**).

Ease of operation, use same equivalent LC/MS software to reduce labor on operation and learning curves (MassLynx).

Informatics: main stream software platform to ensure minimal workload on data handling and integration for efficient process (NuGenesis SDCMS).

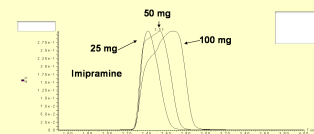
Effect of separation temperature on carbamazepine peaks



Peak shape doesn't change much with increasing temperature compared with RP HPLC separations. However, temperature can still be very helpful with selectivity (see next).

Mobile phase without buffering

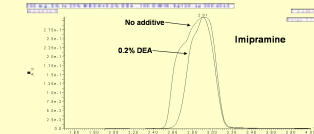
Effect on peak shape as a function of loading



when loading increases, peak shape suffers

Mobile Phase Buffering

Controlling separation under high mass loading



Buffering can help a lot with peak shape under high loading conditions. SFC peak shape becomes much better with adding 0.2%DEA in MeOH.

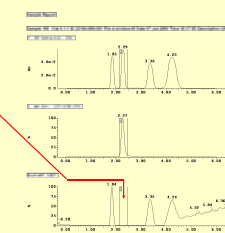
We believe adding a 515 pump & 6-way valve will add significant value by allowing method selection of 6 buffer choices and their concentrations in the separation approach proven in RP-LCMS based systems.

Other Automation Features: Waste UV chromatogram

• Useful in setting collection delay parameters

• Waste UV chromatogram to show compound was collected (not lost). This helps eliminate time consuming discussions about "where did all my compound go? I know I had xxx mg!"

• Convincing nature of data presentation minimizes need for post purification QC for ordinary compounds (95% purity threshold cmpds).



Other Automation Features: ELSD collected mass estimation

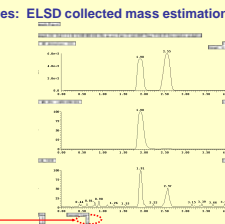
• ELSD Characteristics

• Mass based detection (not concentration)

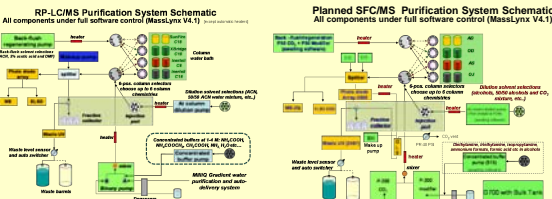
• Fairly analyte independent

• +/- 20% accuracy readily achievable

• Automated inclusion in FractionLynx report



Much of our design philosophy comes from our established approach toward RP-LC/MS based purification



Instrument photo



Key components of SFC/MS system

Gas-Liquid Separator (GLS)

The flow stream passes back pressure regulator at this point

Depressurization of CO₂ occurs at this step

The significantly increased volume of gaseous CO₂ needs to be dealt with, rather than letting it flow into collection tubes, which will cause all sorts of problem

GLS: Minimal CO₂ at collection tip

Most gaseous CO₂ is vented from the top of GLS

Aerosols usually accompanied with this process is mostly eliminated and/or effectively controlled. This ensures minimal sample loss and enhanced safety

GLS: smooth flow into collector

The residual liquid flow is guided through the bottom of the chamber to open-bed fraction collector. Separation pump adjusts with separation gradient to maintain steady liquid flow through the GLS

Open-Bed collection

Collecting in same tubes/facskes used in LC to ensure safety and eliminate complexity for otherwise required customized collection design, workload and cost is lowered significantly, and system robustness is enhanced by ease of operation

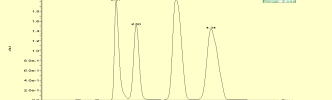
(collecting into EPA tubes)

Gas-Liquid Separator (GLS)

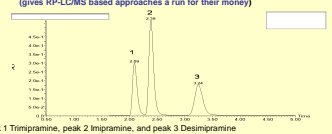


Example 1: Separation cover diversity of samples, neutral, basic and acid!

(well known standards - easy stuff)

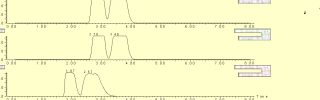


Example 2: Separation of challenging basic drugs: desimipramine, imipramine and trimipramine (gives RP-LCMS based approaches a run for their money)

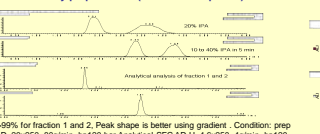


Example 3: Flurbiprofen on AD column (well known chiral example, loading test)

Top and middle: 100 mg and 50 mg injection with 5 to 40% MeOH in 5 min gradient; bottom: 50mg injection and 15% MeOH with isocratic 100g/min and 30x150 mm AD+H column

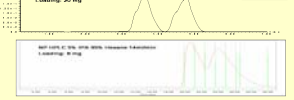


Example 4: Separation and purification of an enantiomeric mixture by prep SFC/MS (in house compound)

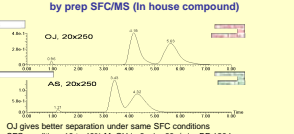


Example 5: Comparison separation and purification of an enantiomeric mixture by prep SFC/MS and NP HPLC (in house compound)

Under SFC conditions, baseline separation in 7 min. with single tube per peak, but NP-HPLC takes 30 min to separate enantiomers partially and requires collection in many tubes.



Example 6: comparison Separation and purification of an enantiomeric mixture on OJ and AS column by prep SFC/MS (in house compound)



Summary

Our gradient SFC/MS based chiral purification platform was setup for short time and several components have not been implemented yet. But preliminary results show that SFC100-MD system works well for chiral and Our new Waters/Thar gradient SFC/MS based chiral purification platform has been setup for only short time and several promised components (pumps) have not yet been implemented. Nevertheless, preliminary results show that SFC100-MD-1 system works well for chiral and achiral separation and purification and is much better than NP HPLC in terms of efficiency (speed and loading). In many regards, our initial impression is that the system works better than we expected.

100 mg/injection has been achieved for both chiral and achiral purification. Analytical data of post purification fractions show that ee is >99% for all enantiomers. We also have successfully implemented additional ELSD and UV detectors into the system. We believe that with addition components, on column dilution systems with CO₂ and modifier, gradient back-flush system, separate buffer delivery sub-system, we will meet all our initial goals: routinely inject >100 mg and yield >10 g per day of high purity (95-99% ee) for any number of compounds at 1-200 with >95% success rate.

With this new hardware, we will continue to experimentally push the envelope on purification production and performance. Of course, as progress is made, we will continue to report it.