Supercritical Fluid Chromatography (SFC) interfaced to cold electrospray MS (and other common detectors), the next, go there first, analytical technique? …and it's green too?

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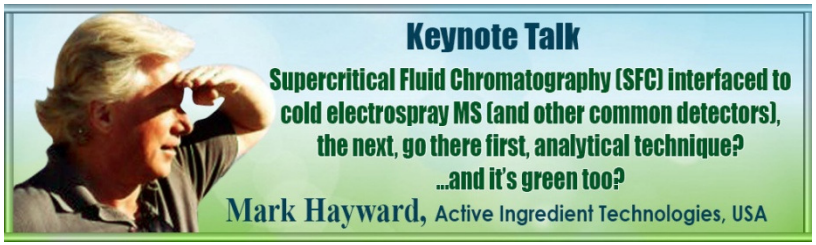
45th Anniversary of American Laboratory



Supercritical Fluid Chromatography (SFC), the next go there first technique!

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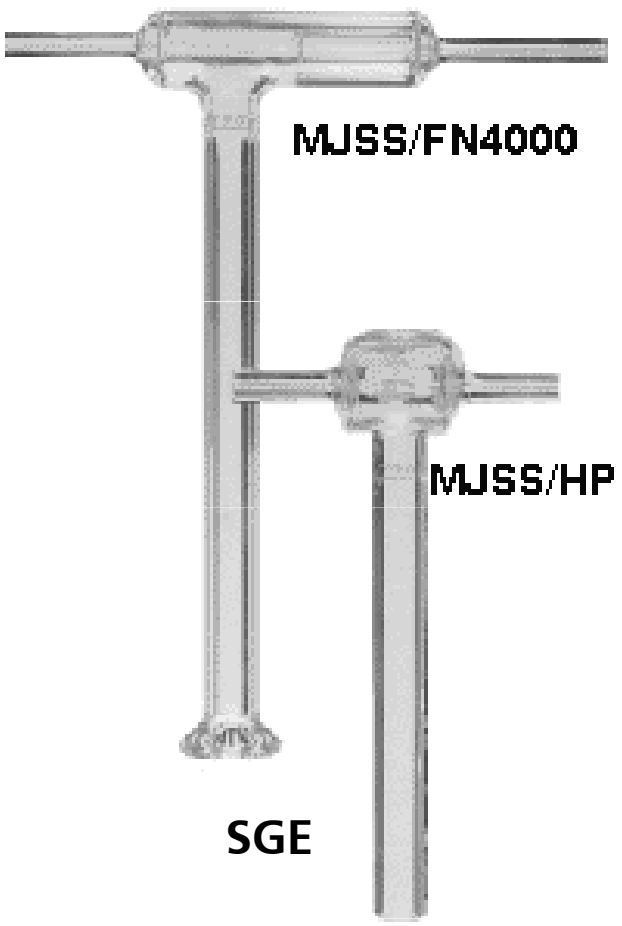
Interfacing chromatography to MS

- The first 5 decades of MS use involved leaking vapors into MS or introduction via a solids probe (often heated)
- Any separations needed were performed offline, prior to sample introduction
- Then, in the 50s and 60s, scientists began to realize the utility of GC separations
- As a result, efforts to interface pressurized flowing streams to the vacuum of MS instruments began to change the ways we use MS

Interfacing separations to MS started with GC/MS

- In the early 60s, the jet separator allowed interfacing packed column GC to MS*
- Capillary column GC largely eliminated need in 70s – 80s (column connected directly to EI/CI ion source)
- In the 80s, a similar approach evolved into particle beam LC/MS interface
- At the same time, Thermospray efforts taught us that that applied voltage was important and that atmospheric pressure ionization was possible. This led to the API LC/MS interfaces that we used in the 90s and to this day.
- Still, we are not done with our MS interfacing efforts: EXAMPLE SFC!



*This was pivotal for the use of GC/MS in environmental applications in the 70s and 80s

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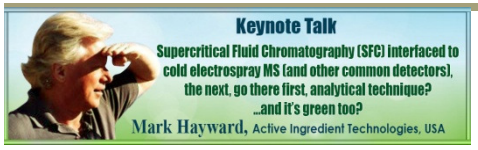
First things first: The value of SFC

- Reverse phase (RP) separations cannot do it all!
  - For polar molecules where differences are in polar parts (often pivotal in chemistry, biology, and biomedical efforts), normal phase (NP) often is most versatile column chromatography approach
  - The potential biology / biomedical need for NP separations may be similar in size to current RP usage for DMPK efforts → Science is telling us we must isolate & measure biomarkers!
- A strong case can be made that SFC is the best way to perform normal phase (NP) chromatography
  - Both NP-LC and SFC can be used for very challenging NP separations, often producing similar separations with the same columns, **but:**
  - While similar outcomes can be achieved, there are significant differences between NP-LC and SFC
  - The primary differences are in the productivity and detection

The value of SFC: productivity

- SFC is much faster than NP-LC because the optimum eluent velocities are much higher (& van Deemter curves flatter)
- Greater analyte solubility in SFC eluent often allows a larger range of mass load than NP-LC
- Perhaps most important – generic gradients in SFC allow rapid method development and focused gradients can achieve stacked injection speeds while simultaneously improving the separation relative to isocratic operation
- We perform many NP-LC & SFC separations and see the sum of the benefits:
  - **Repeated run metrics combining R_s/unit time (3x) & mass load (>2x) suggest SFC is 7 fold more productive (average) than NP-LC**
  - **Simultaneously, SFC requires only one third the method development time**
- **Still, in some regards (trace analysis), SFC is only as good as detectors that can be used effectively with it!**



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The value of SFC: detection

- While SFC is a clear productivity winner in terms of the NP separations, it remains mixed at best with regard to key detection approaches*
  - UV: noisy & noise raises detection limit (≥10x)**
  - ELSD: ditto, but much higher T and less N_2 can help***
  - MS: SFC works better than NP-LC with hexane, but still poor compared RP-LC
    - Particularly poor with most desirable ionization: electrospray****
    - Cold electrospray (ESI) is noisy and sensitivity is down 100x or more relative to RP-LC****
    - APCI works better than ESI when APCI heated to high temperatures*** but produces unwanted fragments and covers limited chemical space
- **In order to reach its full potential, SFC needs to be on par with RP-LC using the 3 detection approaches above**

*R Helmy, CJ Welch, et.al., *Chirality*, 2007, 19, 787.
**TA Berger, BK Berger, J. Chromatogr. A, 2011, 1218, 2320-2326.

***In-house observation

****>90% of all SFC/MS work uses heated APCI

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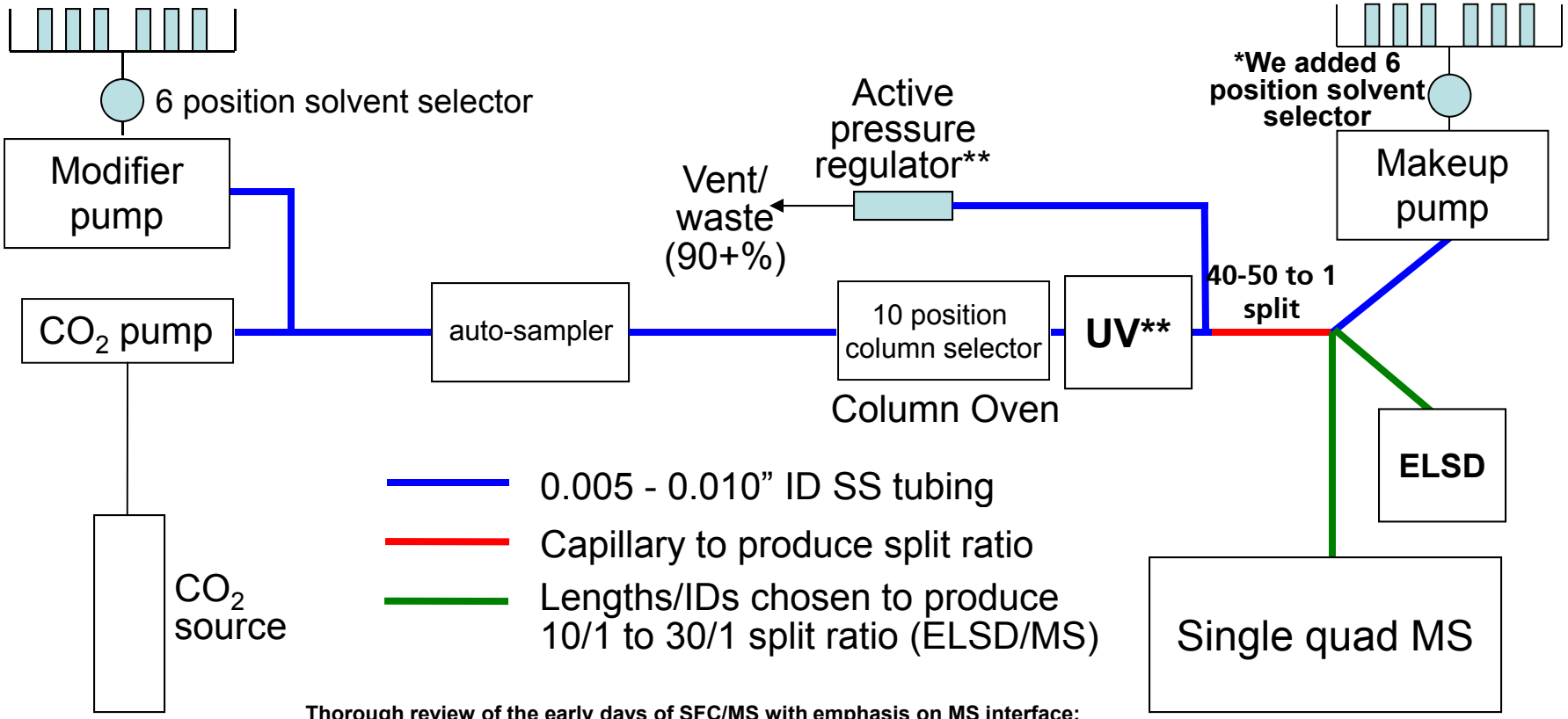
Most common detector interfacing approach for SFC:
our initial approach for a generic SFC system



Thorough review of the early days of SFC/MS with emphasis on MS interface:
M.T. Combs, M. Ashraf-Khorassani, L.T. Taylor *J. Chromatragr. A* 785, **1997**, 85.

Traditional split interface SFC/MS set up:
P.J.R. Sjoberg, K.E. Markides *J. Chromatragr. A* 785, **1997**, 101.
T. Baker, J.D. Pinkston *J. Am. Soc. Mass Spectrom*. 9, **1998**, 498.
D.G. Morgan, K.L. Harbol, N.K. Kitrinos *J. Chromatragr. A* 800, **1998**, 39.
M. Garzotti, M. Hamdan, *J. Chromatogr. B* 770, **2002**, 53.
B. Bolanos, et.al. *Int. J. Mass Spectrom*. 238, **2004**, 85.

SFC/ELSD split interface:
J.D. Pinkston, TR Baker, *J. Am. Soc. Mass Spectrometr,* 9, **1998**, 498.
Z Wang, *Int. Labmate*, Jan **2007, 12-13**.
P Carraud, M Dreux et.al., *J. Chromatogr.*, **1987**, 404, 95.

***Solvent & buffer choice for make up should drive sensitivity**

****UV noise driven by density (RI) changes caused by BPR → pressure & density are important!!!**
TA Berger, BK Berger, J. Chromatogr. A, 2011, 1218, 2320-2326.

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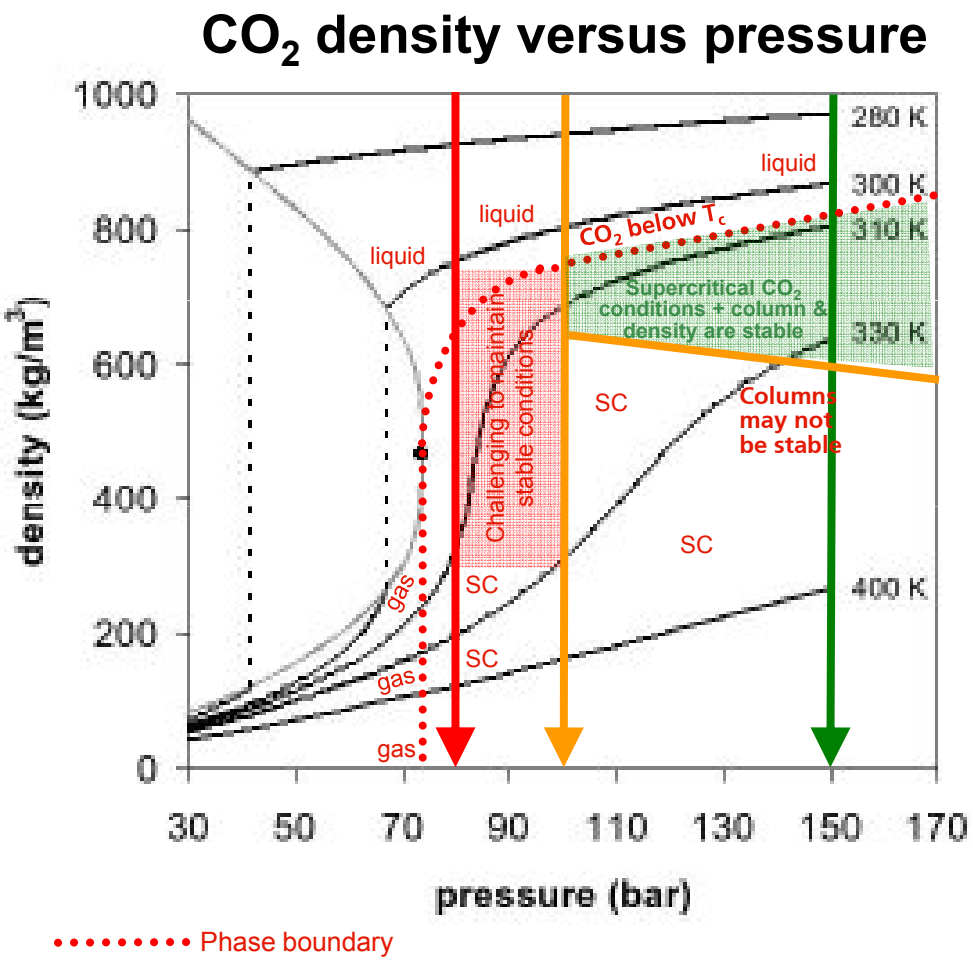
The importance of operating pressure:
making the case for 150+ bar at column exit and UV



- Some consider 80-100 bar post column to be sufficient for SFC operation
- However, density is highly temperature dependent at 80-100 bar (3°C change can result in 2x density change) which results in a high variability in retention times (RTs)
- ±2°C is as well as we can expect to control temperature
- UV noise also driven by density changes caused by BPR cycling (pressure changes)*
- Do we really need active BPRs?**
- **Operation at 150+ bar reduces UV noise* as well as density & RT variation*** across full temperature range where columns are known to be stable (≤60°C) → choose 150+ bar!**

*<u>Agrees with detailed study of UV (RI) baseline</u>:
TA Berger, BK Berger, J. Chromatogr. A, **2011**, 1218, 2320-2326.

**<u>Suggested no BPR needed</u>:
J.D. Pinkston, *Eur.J. Mass Spectrom.* 11, **2005**, 189.
*** <u>Green area corresponds to the B-C boundary in</u>:
Tarafder, A, Guiochon, G., J. Chromatogr. A, **2011**, 1218, 4576-4585.

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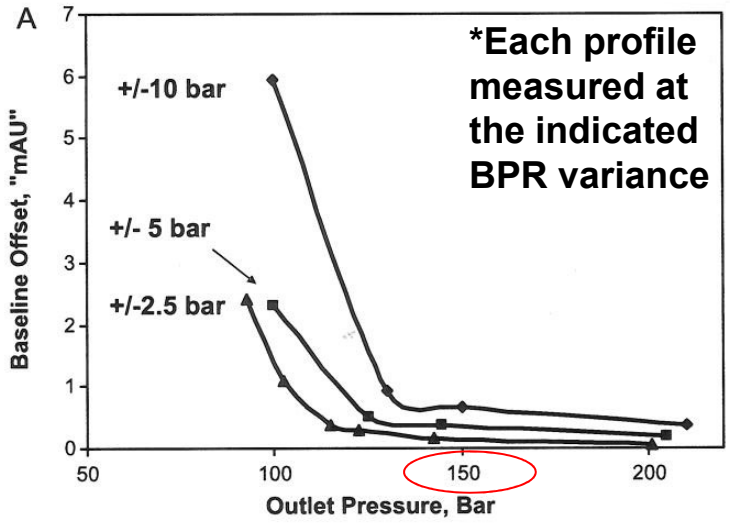
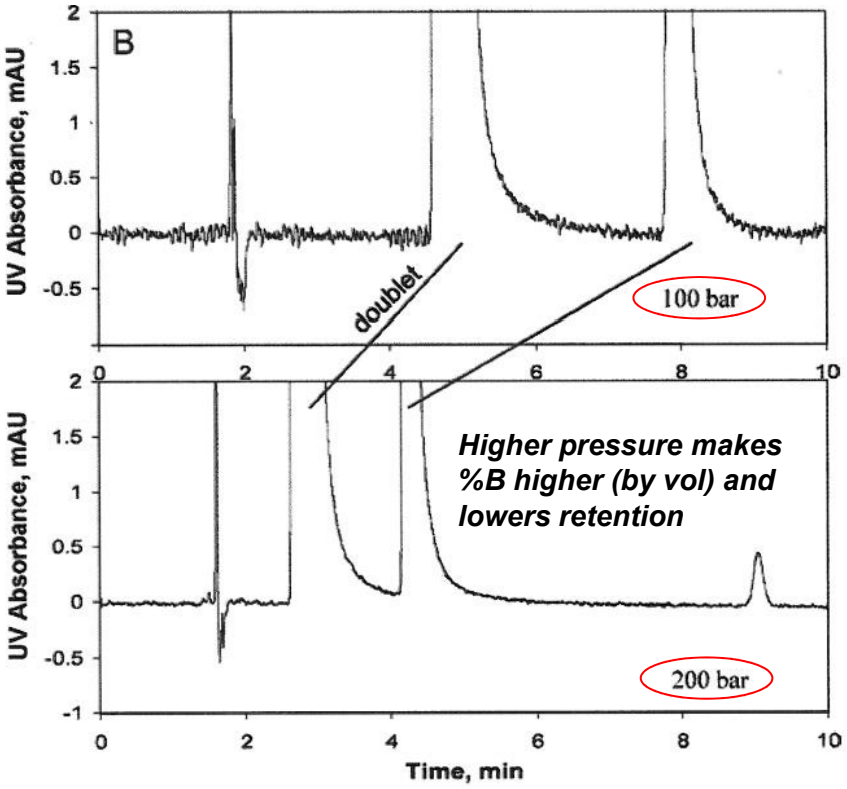
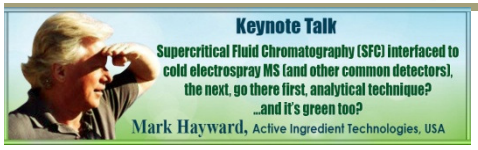
The importance of operating pressure:
with focus on UV detection – noise problem appears to be at least partially due to pressure



- BPR noise flattens out above 130 bar (left)*
- Even with small BPR variance, it's important to have sufficient pressure (right)*
- **Do we need active BPR?****

40°C





*Detailed study of UV (RI) baseline: TA Berger, BK Berger, J. Chromatogr. A, **2011**, 1218, 2320-2326.

Suggested no BPR needed: J.D. Pinkston, *Eur.J. Mass Spectrom.* 11, **2005, 189.

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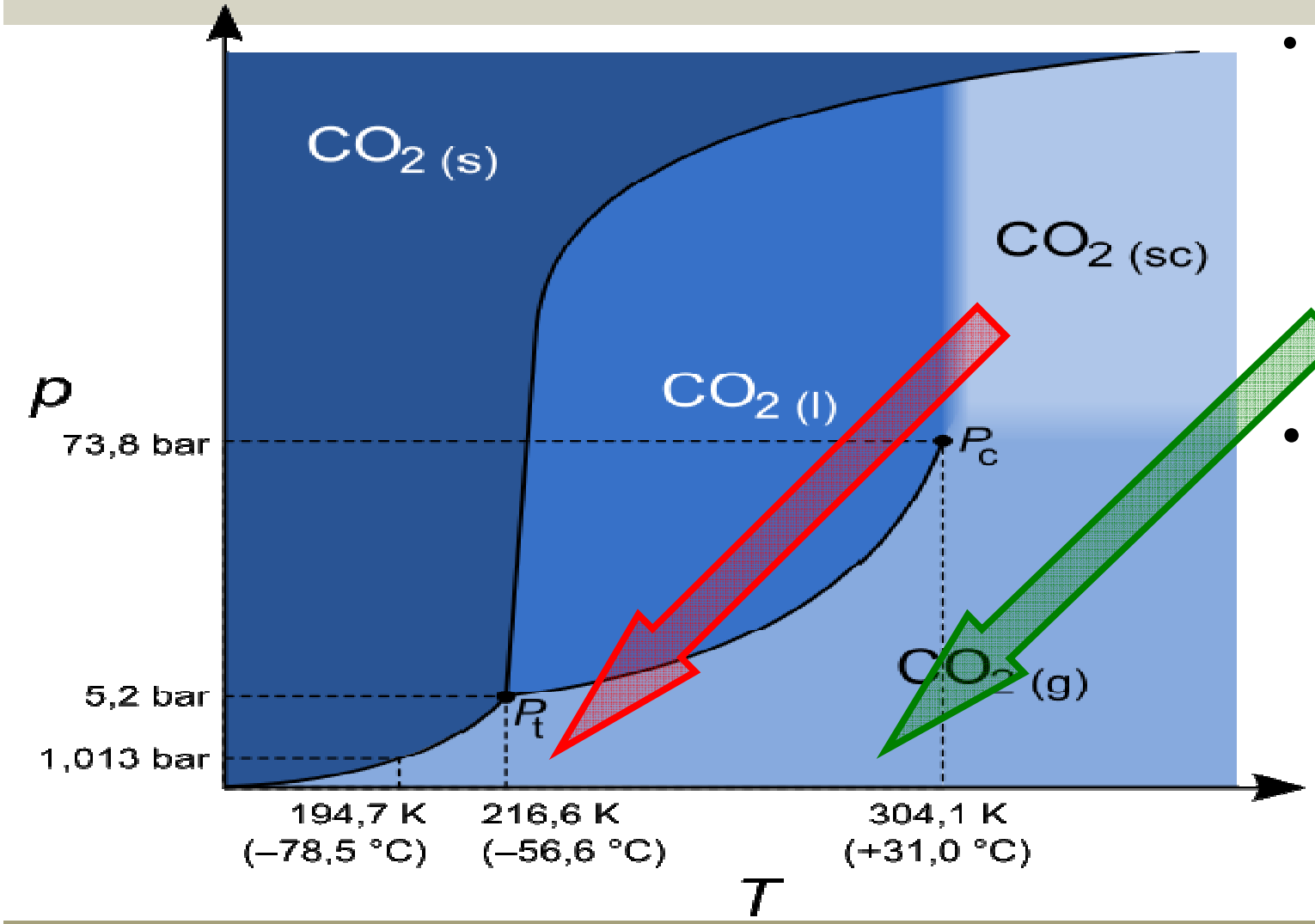
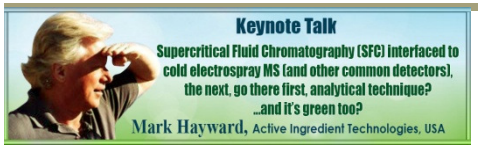
Challenges of interfacing SFC to MS (& ELSD): hard to understand the seemingly contradictory data

- **Splitting flow & using make up solvent (classic approach)**
  - Despite eluent being mostly gas at AP, full flow (1-5 mL/min) into the source (ESI, APCI) hasn't worked well (especially ESI: high background, low response)
    - Sample blown away?
    - Lower flow, 5-50 μL/min alcohol from column seems to provide better sensitivity
  - Conventional Wisdom: Use APCI & make up flow (200-400 μL/min) of alcohol improves signal stability and sensitivity (via dilution of amine buffer?)
- **CO_2 is different (not as inert as N_2)**
  - The use of flow injection (FI) on a LC/MS is not a viable approach toward tuning / optimizing make up solvent composition
  - Presence / absence of buffer does not correlate well with SFC sensitivity
  - FI/MS under LC/MS conditions (identical to SFC except no CO_2) often suggests acetonitrile as most sensitive make up solvent
  - In the presence of CO_2, alcohols for make up flow usually provide better sensitivity (MS & ELSD)
  - SFC sensitivity seems to correlate with physical properties (viscosity), not chemical properties (sensitivity trend: IPA > EtOH > MeOH > ACN)
  - **Perhaps the real issue is phase separation upon expansion of CO_2**

Avoiding phase separation: a working hypothesis for ELSD & MS interfacing with SFC



- **Going to atmospheric pressure the usual way (near T_c) results in cold CO_2 liquid causing phase separation**
- **Increasing temperature allows expansion without phase separation (SC → gas, SC already gas like)**



Starting expansion pressure will define temperature needed

Again, choosing column pressure is an impactful decision

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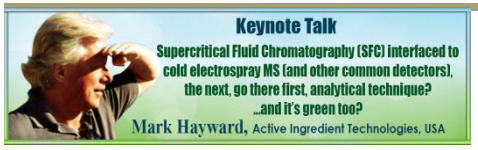
Addressing the phase separation hypothesis to improve noise and sensitivity in ELSD & MS

- Need improve sample utilization
  - All eluent to detectors instead of majority of sample to waste via BPR
- Both temperature and pressure seem to be likely to be important for all detectors
  - Need to actively heat flowing eluent stream (gradients)
    → Selerity CaloraTherm heater (wide range of T)
  - Also, may need to cool
    → Selerity CaloraTherm Peltier (heat/cool narrow range of T)

- Need to minimize pressure variation from BPR
- Proposed solution: combine preheating/cooling with fixed restrictor (instead of BPR)* held at 150 bar**



*__Suggested no BPR needed__:
J.D. Pinkston, *Eur.J. Mass Spectrom.* 11, **2005**, 189.

**__From density vs. pressure data and__:
TA Berger, BK Berger, J. Chromatogr. A, **2011**, 1218, 2320-2326.

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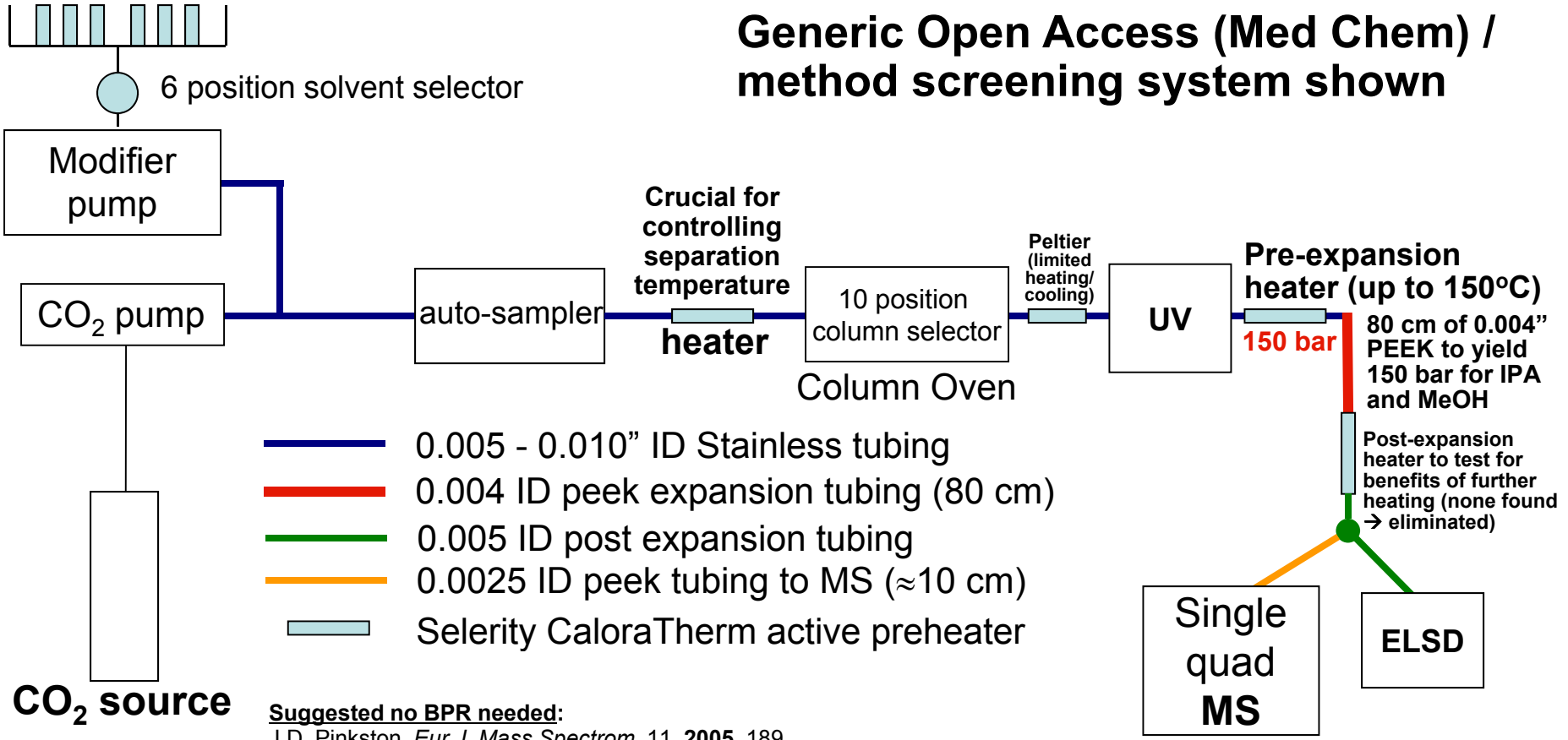
An alternative interface of SFC to detectors



Generic Open Access (Med Chem) / method screening system shown

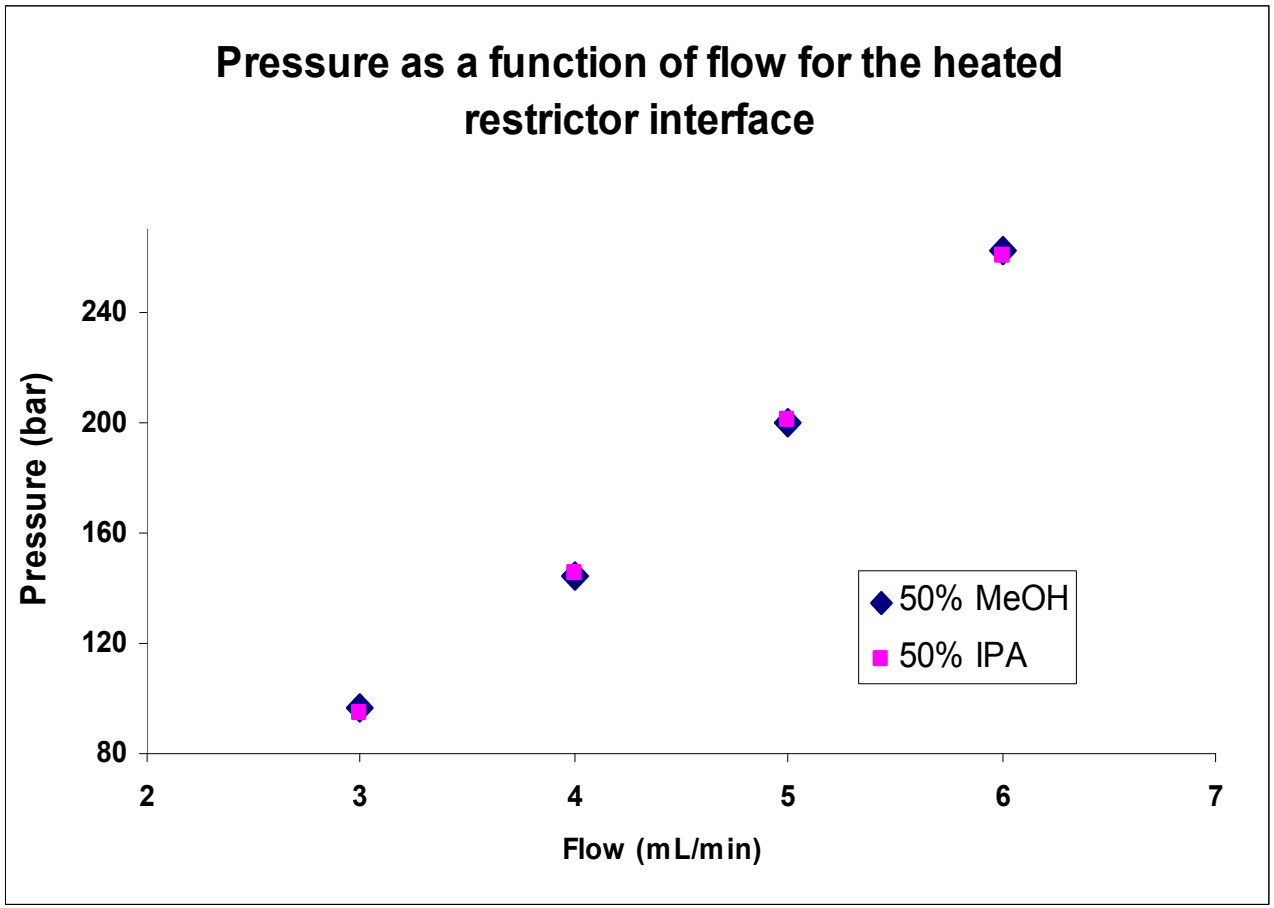
Suggested no BPR needed:
J.D. Pinkston, *Eur.J. Mass Spectrom.* 11, **2005**, 189.
Full flow into heated APPI, ESI, APCI MS sources with good results:
R.A. Coe, J.O. Rathe, J.W. Lee *J. Pharm. Biomed. Anal.* 42, **2006**, 573.
Noted importance of temperature for MS interface:
F Sadoun, H. Virlizer, P.J. Arpino *J. Chromatogr.* 647, **1997**, 351.

For high sensitivity bioanalysis, eliminate UV / ELSD and replace MS with triple quad MS/MS (full flow from restrictor into MS source)

Initial characterization of fixed restrictor

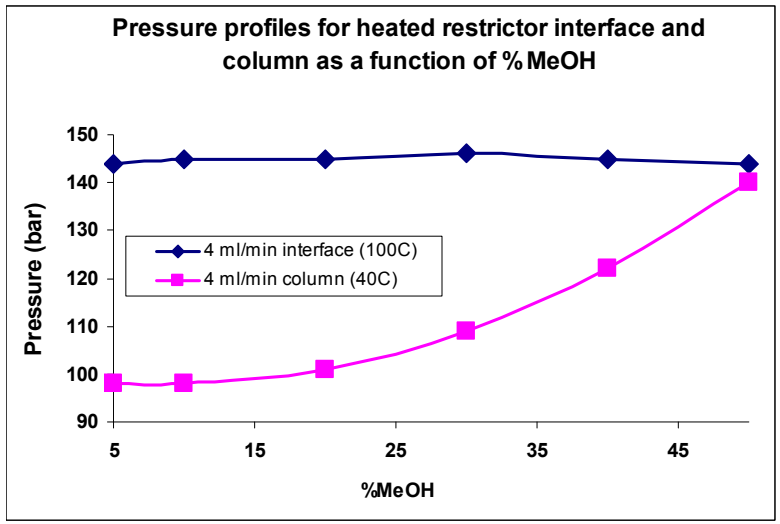
- Initially thought it would be more complicated than it turned out to be because we started with conditions far from optimal (pressure and temperature too low)
- Anticipated that multiple fixed restrictors would be required to adapt to:
  - Different modifier viscosities
  - Different percentages of modifier
- Turned out to be much simpler because optimal expansion conditions (temperature, pressure) occur where the CO_2 / modifier mixture is supercritical and defining pressure is easier to achieve than initially expected
- Won't bother showing all the ineffective conditions

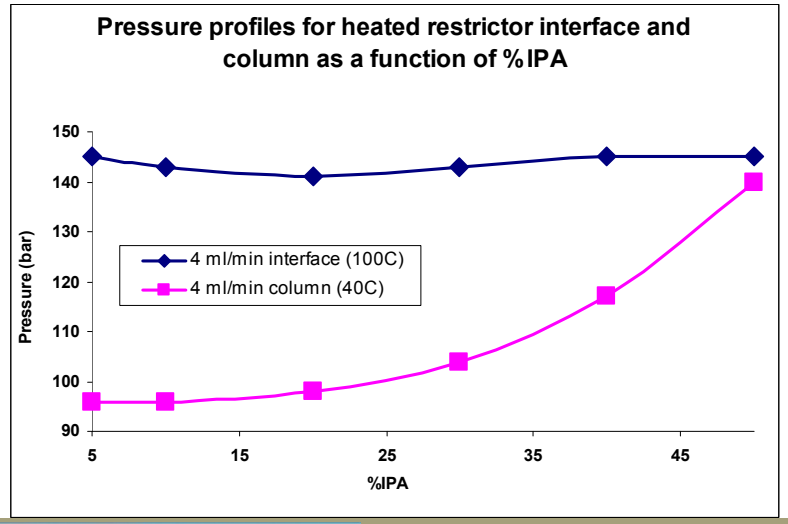
Characterization of fixed restrictor



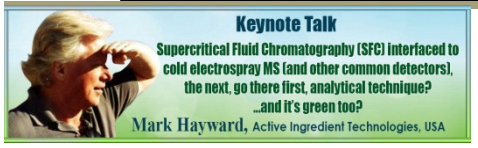
- 0.004” ID PEEK tubing – 80 cm long with eluent entering at 100°C
- 4 ml/min very close to optimum velocity for most separations and gives target pressure of 150 bar
- Does not follow Darcy’s law (turbulent flow), which is not surprising given (CO_2 SC) high Reynolds number ($Re = 10^4 - 10^5$)

Characterization of fixed restrictor





- Data shown for column 150 mm in length with 3 µm particles without MS & ELSD nebulizers* (note: we use only 100-150 mm columns with 3 or 5 µm particles)
- Restrictor pressure constant for all ordinary modifiers (MeOH, EtOH, IPA) across normal modifier range (5-50%)
- Variation in restrictor pressure is due to pump pulsing (not observed with column as it acts as a pulse dampener)
- At 100°C and pressure ≈150 bar, ordinary SFC eluent behaves in supercritical like manner
- Under ordinary separation conditions in column (35-60°C), eluent does not behave in supercritical like manner
- Conclusion: if operating at fixed flow (4 ml/min chosen), a single fixed restrictor can be employed for all other conditions**



*MS / ELSD nebulizers add another 6-7 bar post restrictor

**Temperature can be used to fine tune optimum velocity through column

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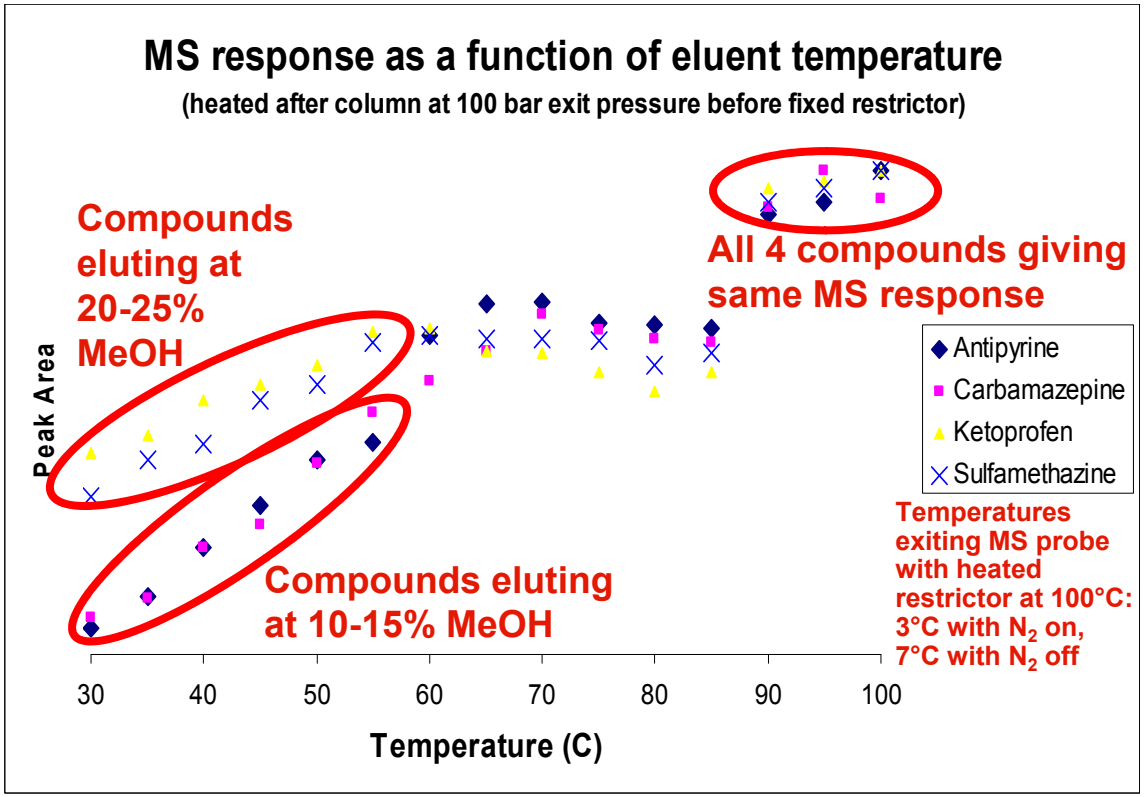
Initial characterization of heating

- Initially thought it would be simpler than it turned out to be because we increased temperature and immediately saw improvement at relatively low temperatures
  - Used CO_2 refrigeration data suggesting 80 bar to AP results in 40°C drop (temperature drop much bigger)
  - Started with post column pressure too low
    - 80 bar data not sufficiently reproducible
    - 100 bar data reproducible at low column temperature (35-40°C), but we frequently go up to 60°C and needed still higher pressure
- Ultimately found we needed to go to even higher temperature
  - Got Selerity to make a higher temperature version of CaloraTherm
- Started with our biggest initial objective → understand MS
  - To prove that issues with MS interfacing are physical (phase changes, i.e. CO_2 is inert gas) and not chemical (CO_2 can be reactive)

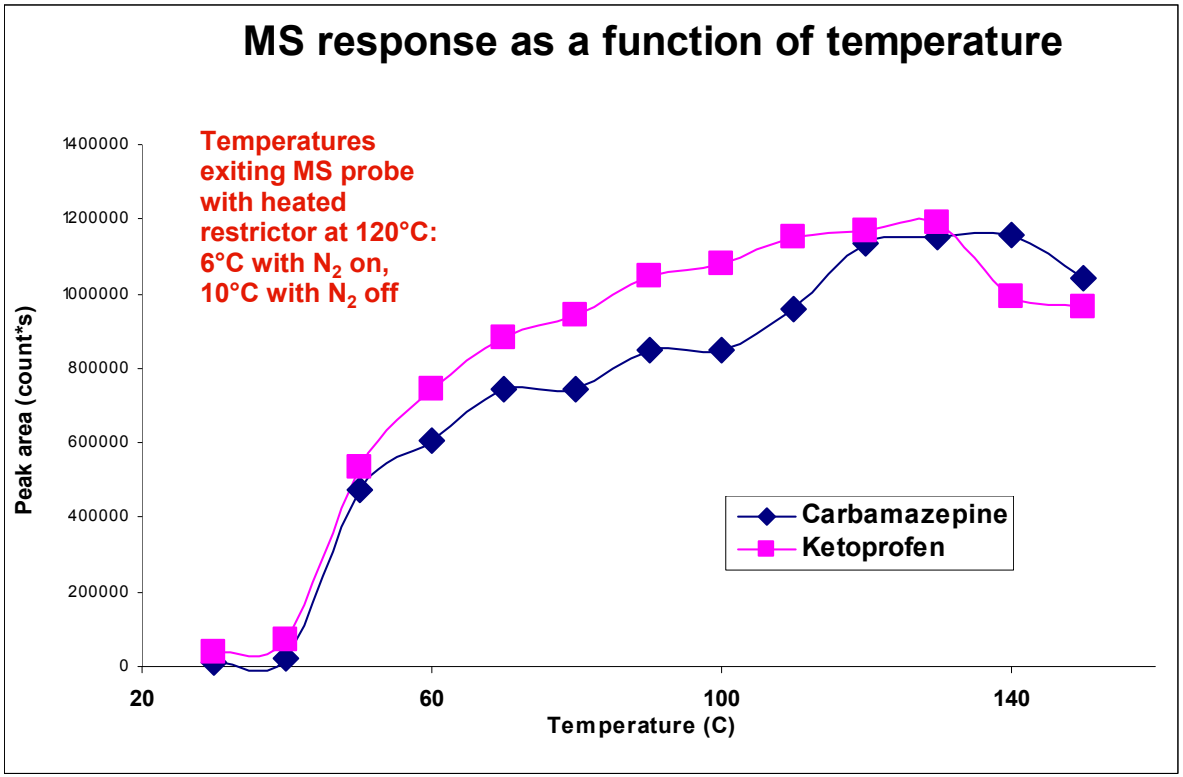
Characterization of heating
using MS detection



Data strongly supports phase separation during expansion to AP hypothesis, but still begs the question: What happens at higher temperature? Optimum conditions not yet found…

- 4 compounds with concentration normalized to give same MS response (FI/MS) at apparent SFC flow rates for MeOH (gradient)
- If SFC/MS conditions can be found where all 4 give same MS peak area, then CO_2 may be inert gas
- If MS peak area follows viscosity, then we have confirmation data
- Indeed, CO_2 appears to be inert (≥90°C) and MS sensitivity does follow viscosity (<60°C)!

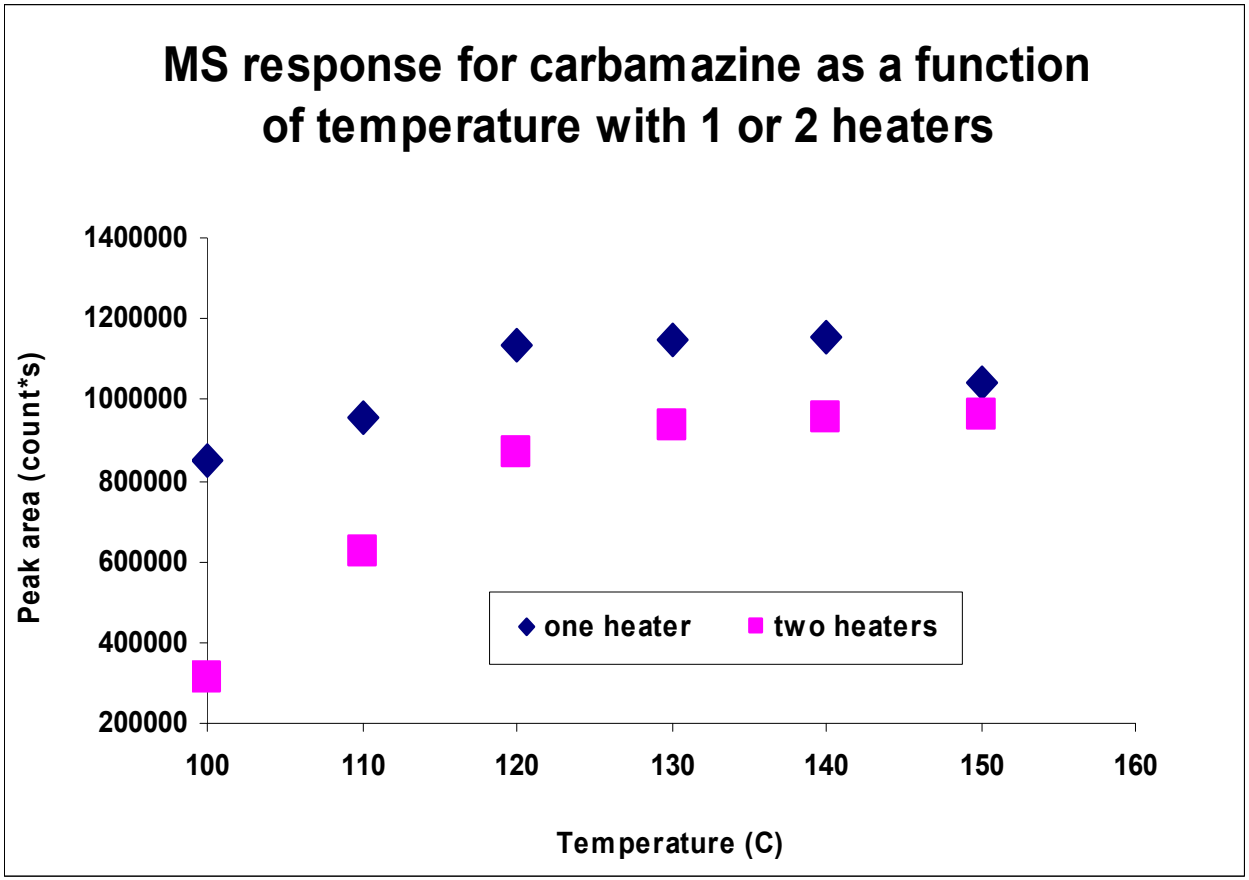
Characterization of heating: higher T
using MS detection



There is some small but significant benefit in using the higher temperature version of the CaloraTherm heater

- 2 of the previous 4 compounds with concentration normalized but now at 150 bar
- Apparent maximum in MS response 130°C
- Shape rise in response seems to occur at lower T when starting from higher P and gives a wide acceptable range of temperatures to operate
- Same viscosity trend seen at lower temperatures

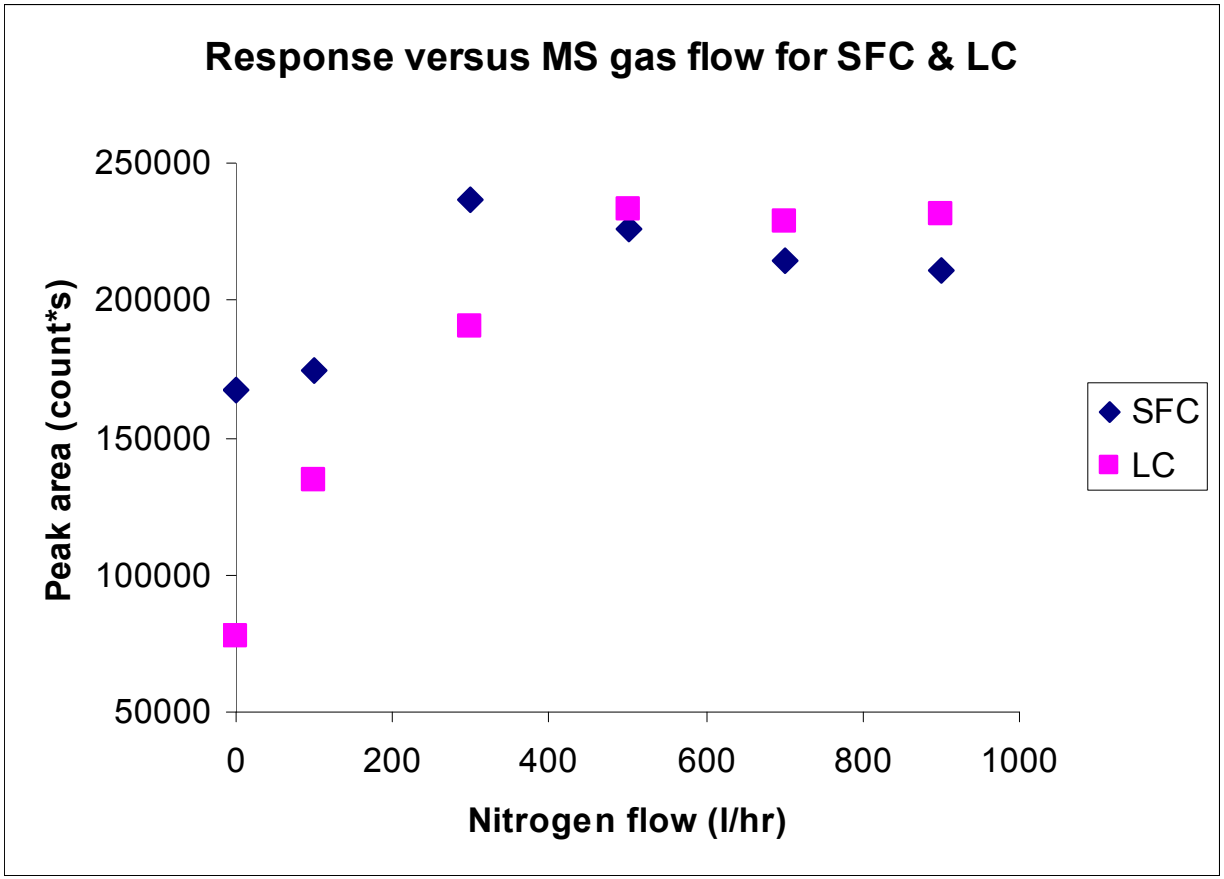
Characterization of heating: 2 heaters
One pre-CO_2 expansion, one post expansion



Using MS detection

- Many combinations of temperatures tested
  - First heater low, second higher
  - First high, second lower
  - Positive and negative fixed offsets
  - Both the same
- Selected data that best represents overall picture (both same):
  - In all cases, one heater works better than two
  - In all cases, heating before expansion works better than after
- Differences are even bigger at lower T

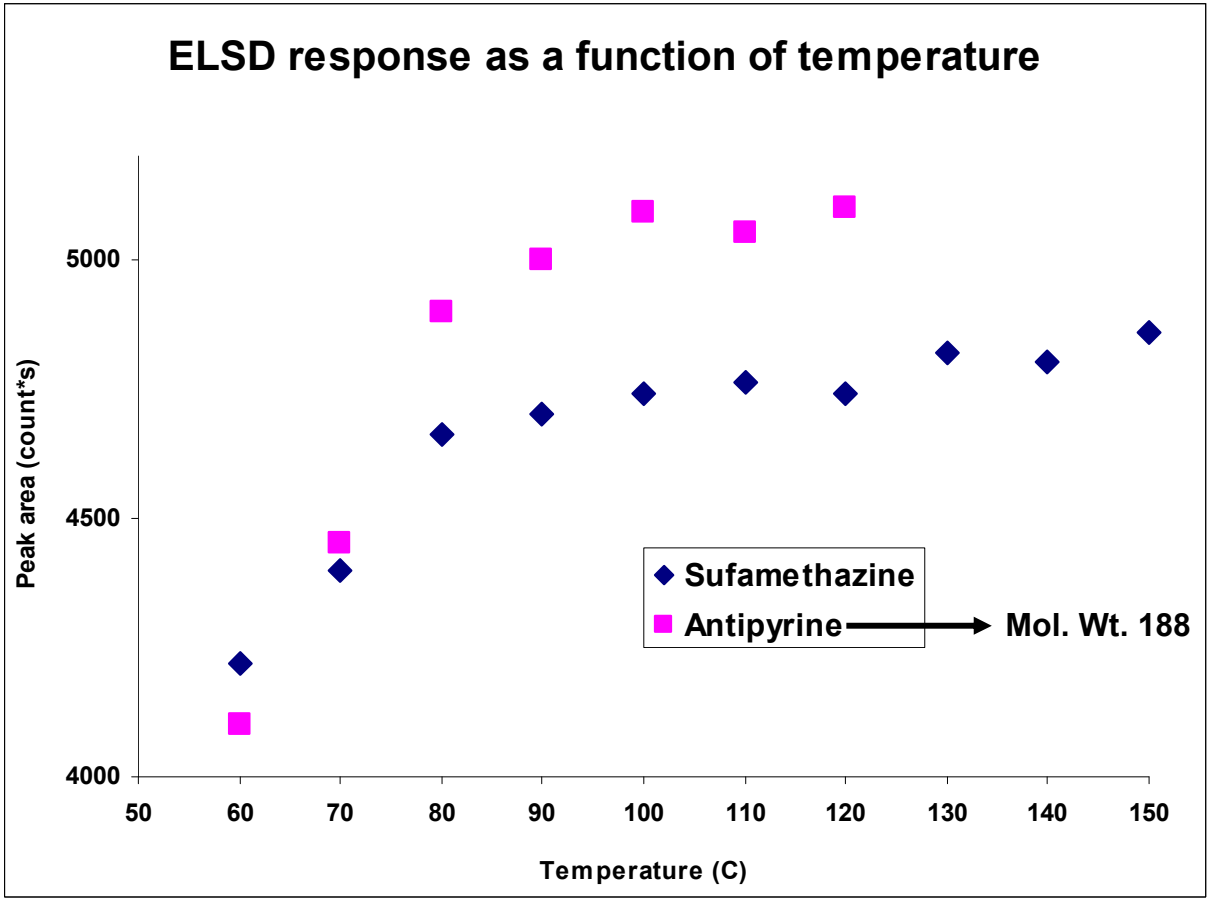
Characterization of heating:
need for N_2 with MS (in addition to 20 l/hr CO_2)



- Sulfamethazine SFC/MS (w/ 150 bar fixed restrictor 120°C) and RP-LC/MS (200 µl/min into source)
- Both using Waters 3100 MS
- Optimum 500 l/hr for RP-LC/MS lowered to 300 l/hr for SFC/MS
- Less effect on SFC/MS at lower flow → 20 l/hr CO_2 already doing some but not all nebulization
- SFC looking comparable to LC for MS detection!

Gas load from SFC CO_2 helps nebulization and allows slightly less N_2 flow

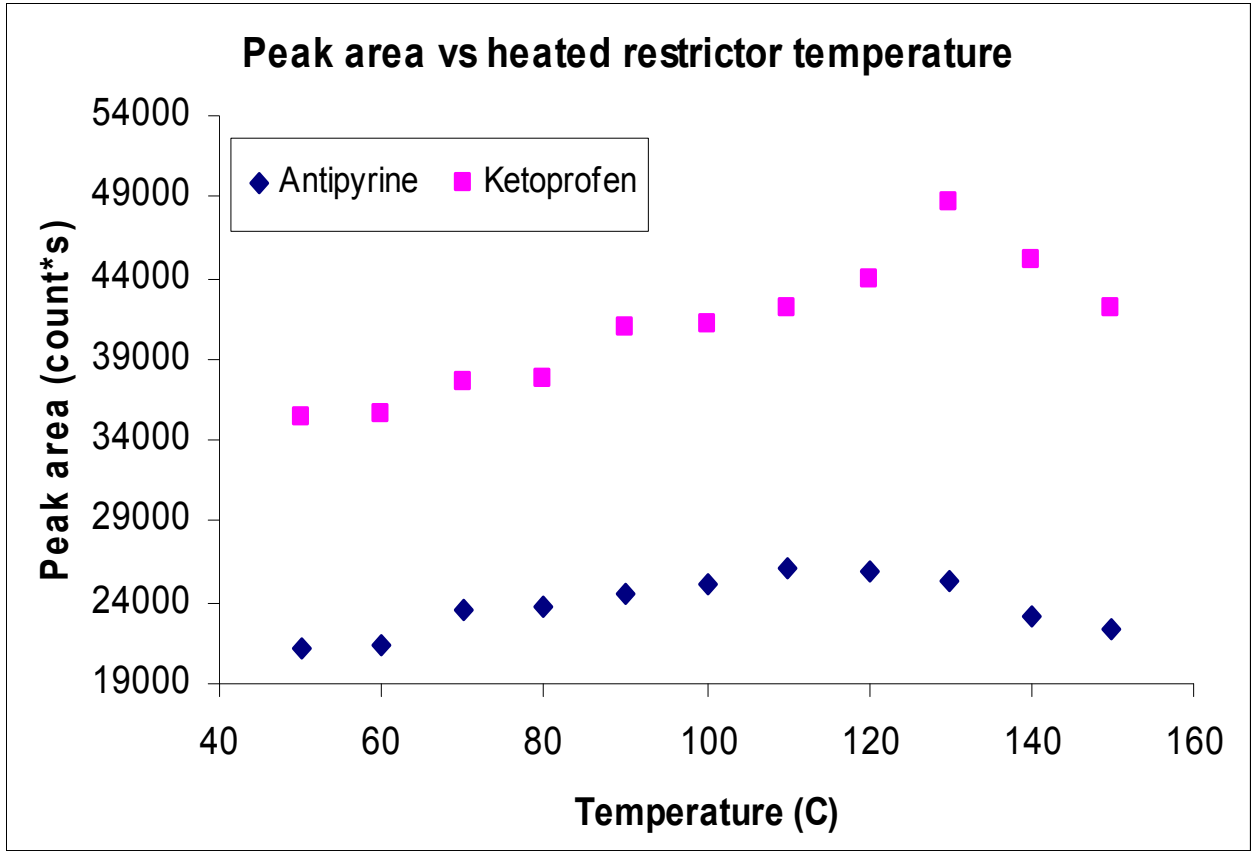
Characterization of heating: ELSD



Waters 2424 ELSD optimal settings:
RP-HPLC – N_2 pressure 60 psi – nebulizer temperature 60°C
SFC (heated restrictor @120°C) – N_2 pressure 50 psi – nebulizer temperature 35°C

- 150 bar at column exit and "normal" ELSD conditions for RP-HPLC (T & P for N_2 in ELSD)
- Higher T helps with benefits leveling off above 100°C
- At still higher T, particle size of low mol. wt. compounds are smaller than can be seen by ELSD (still observed by MS)
- Greater tolerance of lower T at ELSD nebulizer while maintaining sensitivity

Characterization of heating: ELSD
second iteration with ELSD optimized for SFC



Initial impression: ELSD with SFC looks surprisingly good relative to ELSD with RP-HPLC (more examples later)

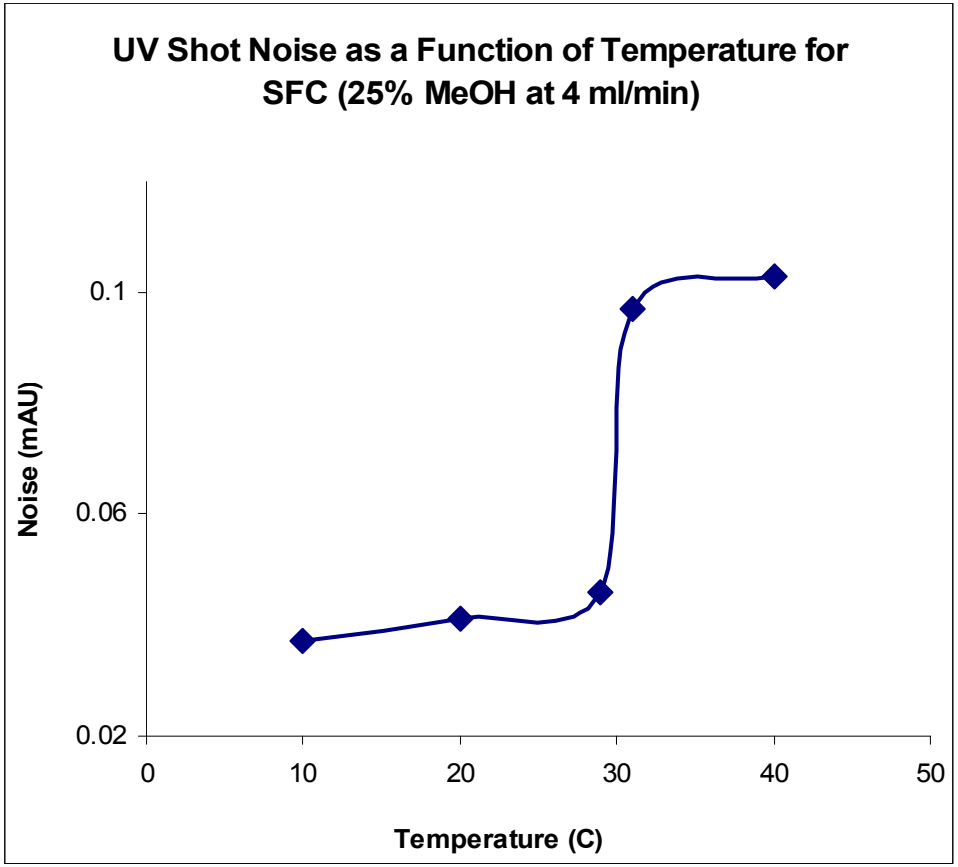
- 150 bar at column exit and new SFC based ELSD conditions (**N_2 pressure 50 psi – nebulizer temperature 35°C**)
- Useable fixed restrictor temperature extended upward to match ELSD and MS optimal conditions
- No more loss of low mol. wt. compounds in ELSD
- Added benefit: much greater sensitivity – antipyrine up 5 fold & dynamic range 10^3

If temperature (avoiding phase separation) helps MS & ELSD so much, can it help UV?

- Pursued hypothesis that CO_2 (sc) mixed with alcohol (liq) at ordinary column T (35-60°C) is mixed phases (sc & liq)
  - Applied heat (40-120°C) before UV to shift mixture to sc conditions and noise (high freq) went up with T
  - Conclusion: no significant phase separation above P_c & T_c
- UV absorption is known to be shot noise limited coming mostly from sample (no benefit in FT, dynamic range much lower than PT [10^4 vs. 10^7])
  - In case of SFC, shot noise comes mostly from most abundant component, i.e. the eluent (CO_2 & alcohol)
  - Lower T should always lower shot noise
  - Lower T for UV known to lower noise for LC
- Formed new hypothesis that SFC-UV would work better as a cool liquid and set out to test it experimentally
  - Used Peltier before UV to reduce shot noise

Reduced temperature UV detection: following the noise

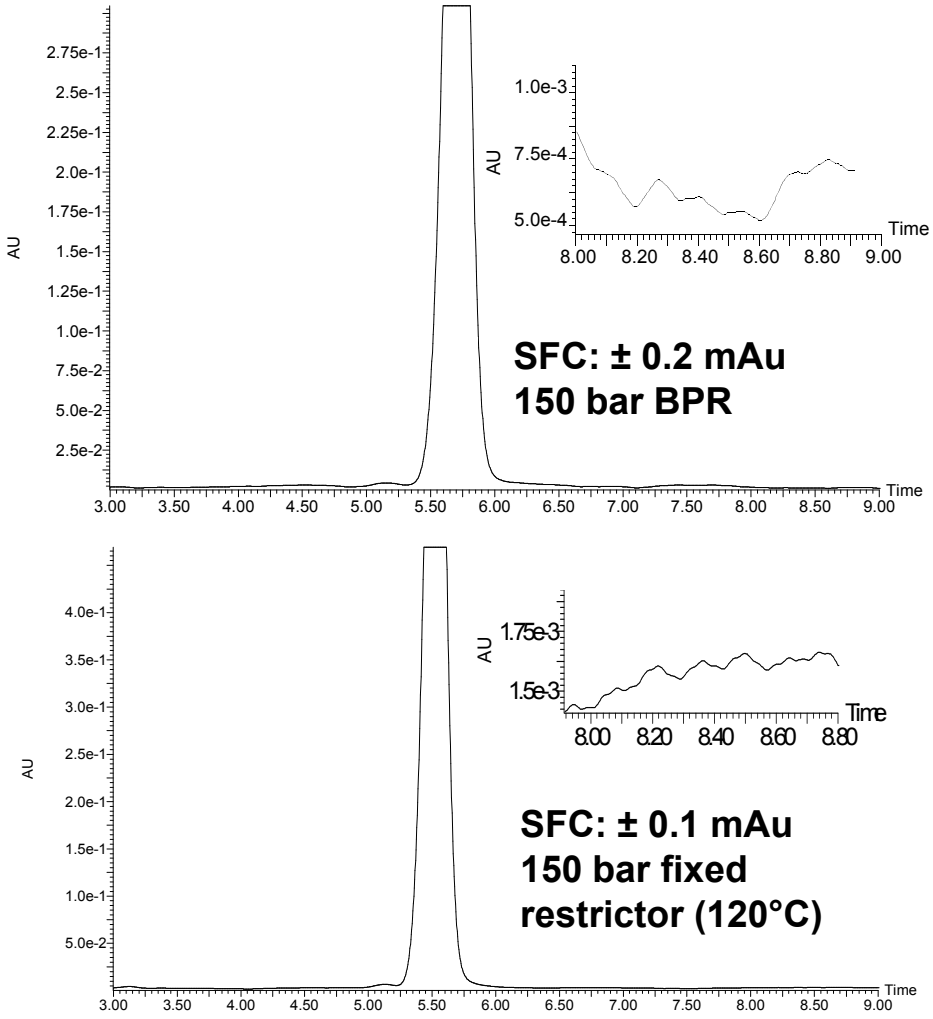
- Peltier used to control temperature of eluent stream prior to UV (temperature range limited)
- Data suggests liquid state produces lower noise
- Noise levels still not as low as LC, but it is getting close
- In theory, if eluent stream and UV detector could be cooled further, equivalence between SFC and LC may be achieved



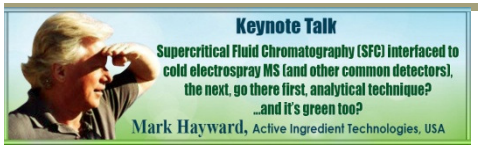
Critical examples comparing SFC with both interfaces and RP-HPLC with all 3 detectors

- UV, MS, and ELSD compared for:
  - SFC with traditional split / makeup interface
  - SFC with heated fixed restrictor interface
  - HPLC performed in the usual ways
- Emphasis placed on sensitivity, noise, and dynamic range
- Goals:
  - Compare BPR / split interface with heated fixed restrictor interface for SFC
  - Establish recommended conditions for heated fixed restrictor interface
  - Compare SFC with RP-HPLC to evaluate if SFC detection is on par with RP-HPLC

UV detection: BPR & fixed restrictor



- Sulfamethazine SFC at 40°C (Waters/Thar FDM & 2998 PDA)
- Tested at 150 bar with BPR and fixed restrictor and no temperature control between column oven and UV detector
- Noise ± 0.2 mAu with BPR and ± 0.1 mAu fixed restrictor
- Noise still 4x higher than Berger data at 200 bar* but cooling achieves near equivalence
- Perhaps 200-300 bar pressure needed to get to lowest noise levels possible* and cooling seems likely to provide further help



*TA Berger, BK Berger, J. Chromatogr. A, **2011**, 1218, 2320-2326.

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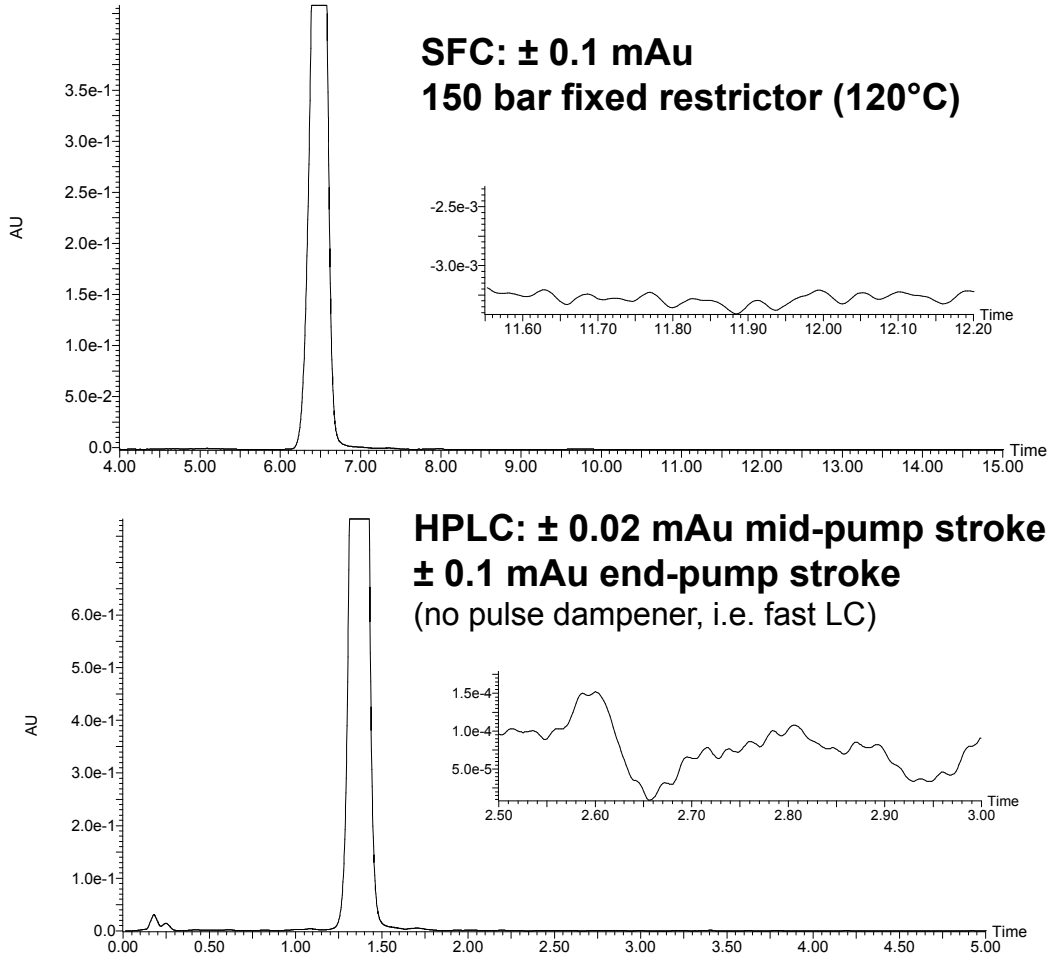
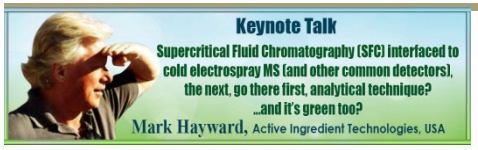
UV detection: SFC & RP-LC



SFC and fast RP-LC nearly equivalent for UV detection
Slower pulse dampened LC can go to 5x lower conc

- Sulfamethazine SFC at 40°C (Waters/Thar FDM & 2998 PDA @150 bar–fixed restrictor w/ high pressure cell) and RP-LC (Waters 1525 & 2998 PDA @AP w/ low pressure cell)
- Filter: 1 s for both
- Cell 10 mm and 9.3 µl for both
- Noise due to RI change still 5x higher for SFC compared to LC with pulse dampener
- LC pump pulse noise (fast LC, no dampener) about the same amplitude as SFC RI change noise, but lower frequency
- LC pulse noise can be removed (but costs time)



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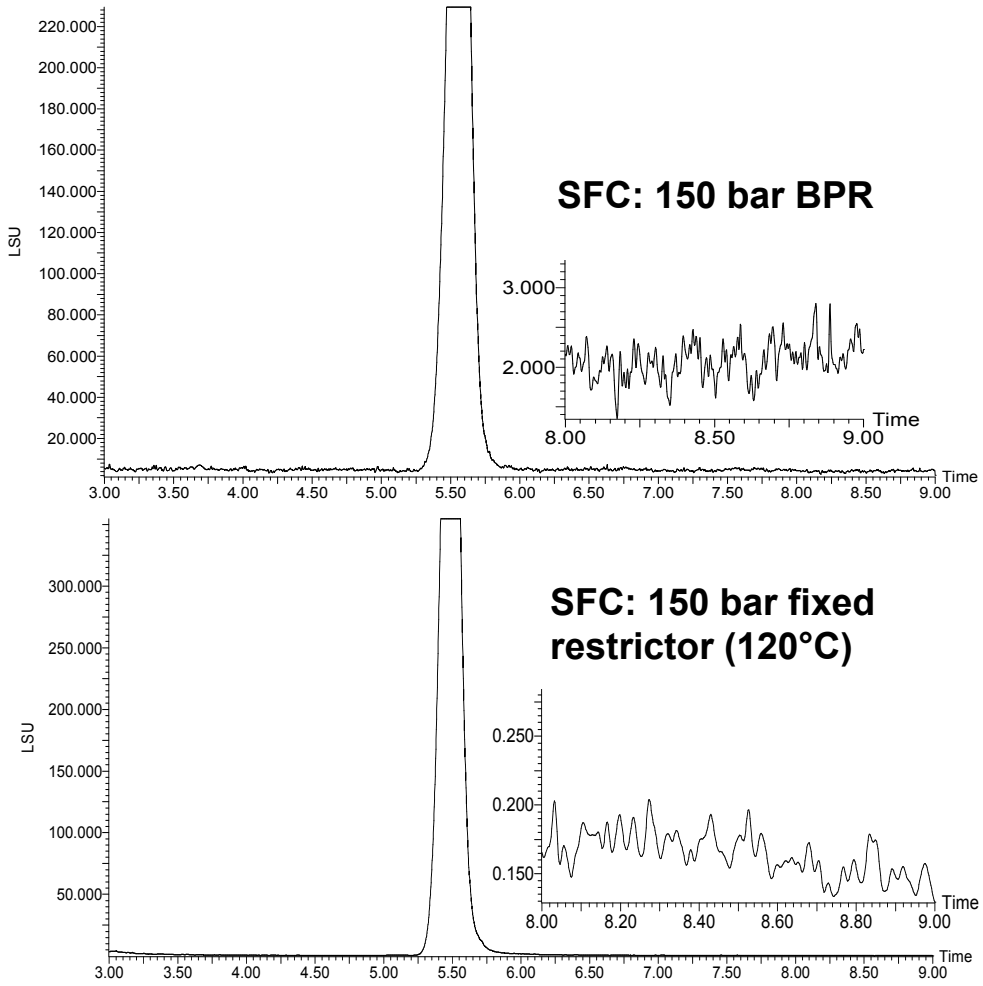
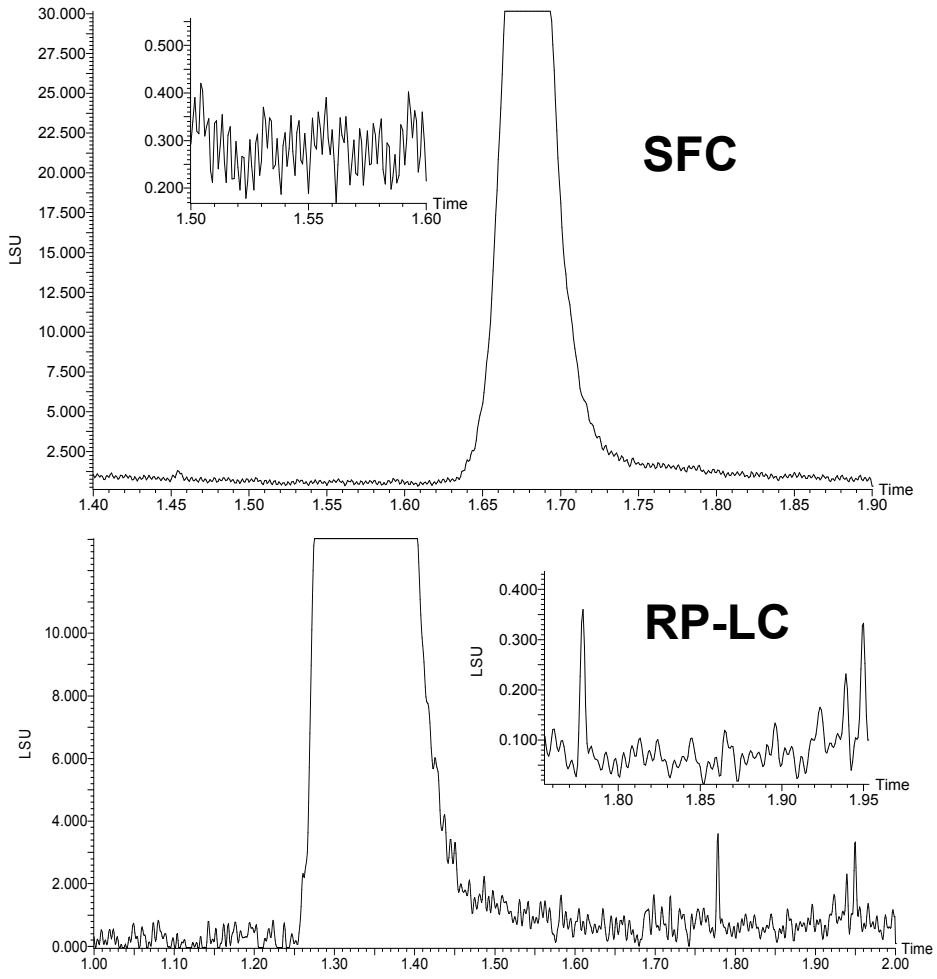
ELSD detection: BPR & fixed restrictor



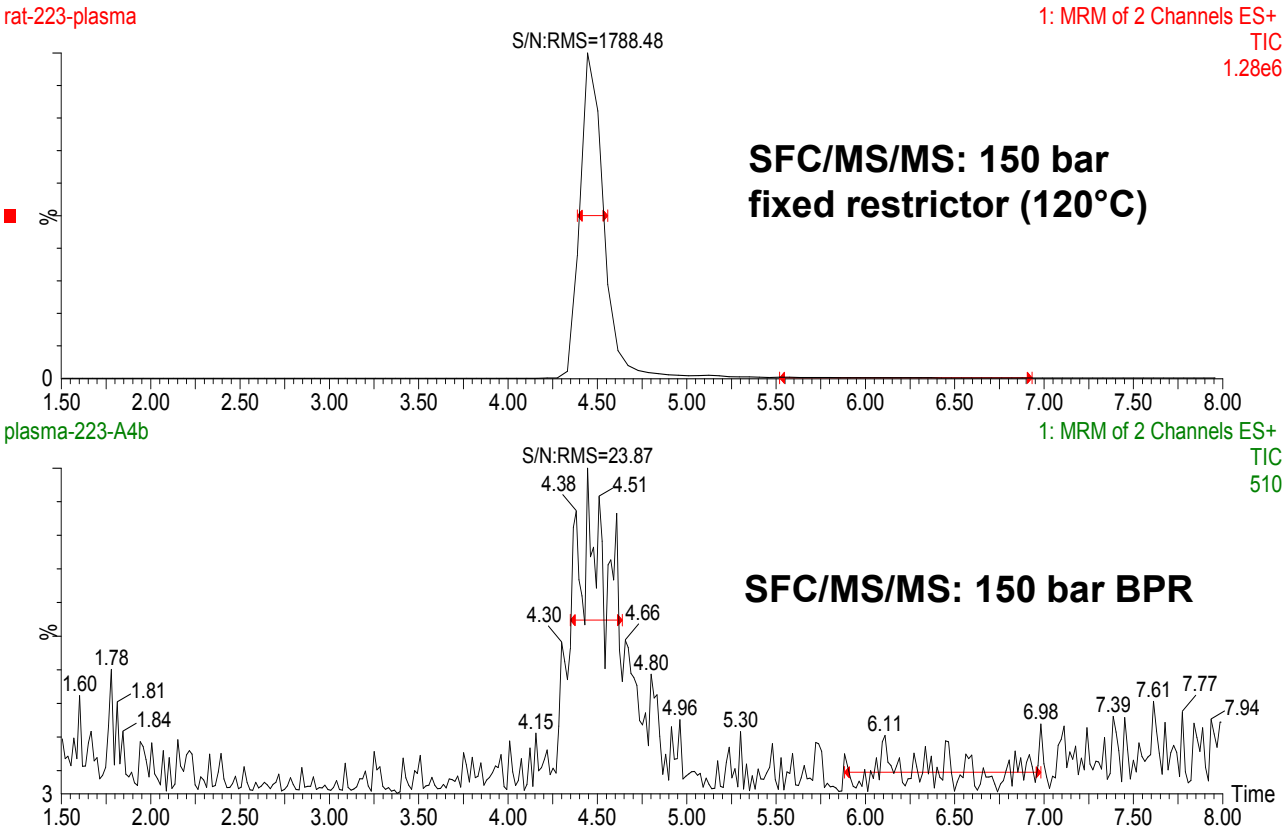
- Sulfamethazine SFC (Waters/Thar FDM & 2424 ELSD)
- Tested at 150 bar with BPR and fixed restrictor + heat
- 20x less noise for fixed restrictor + heat
- 2x greater sensitivity for fixed restrictor + heat

ELSD detection: SFC & RP-LC



- Sulfamethazine SFC (Waters/Thar FDM & 2424 ELSD heated fixed restrictor and RP-LC (Waters 1525 & same 2424 ELSD)
- Filter: 1 s for both
- Noise roughly 1.5x higher for RP-LC
- Sensitivity (response ratio) 3-5x higher for SFC
- Dynamic range 10^3 for SFC and 10^2 for RP-LC
- ELSD clearly works better with SFC relative to RP-LC

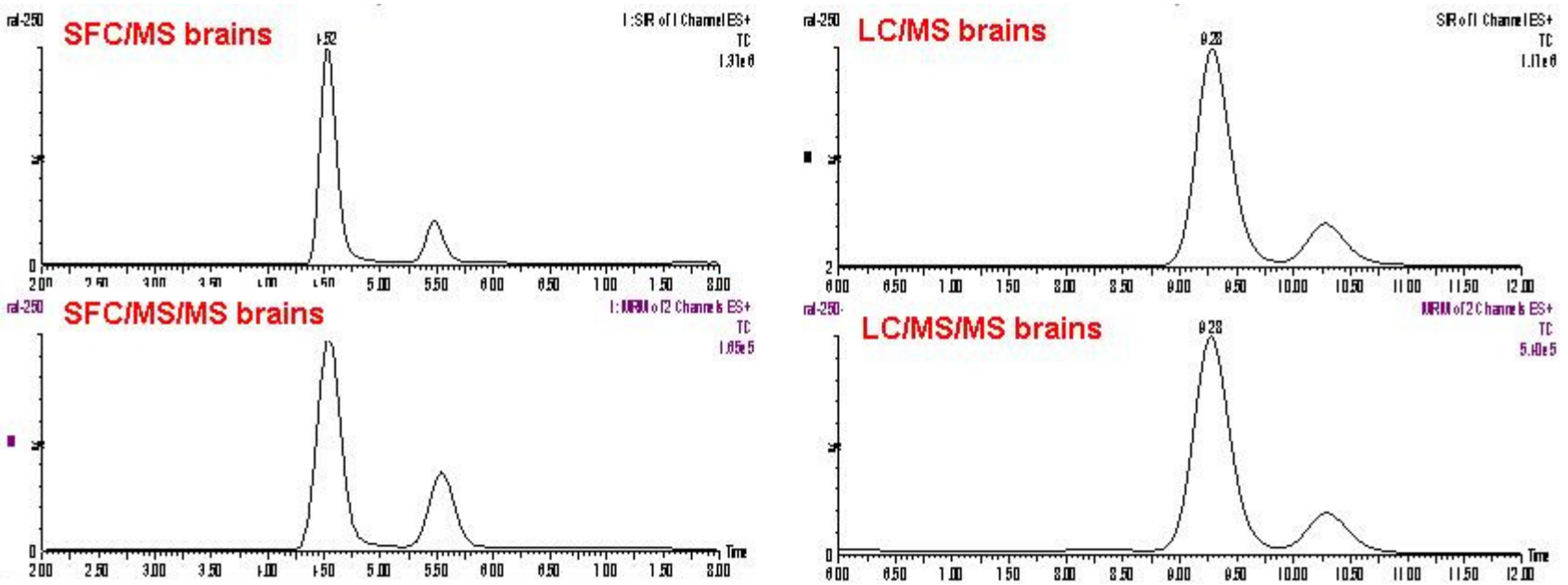
MS detection: BPR & fixed restrictor



- Plasma bioanalysis of drug at 40 ng/ml with Waters/Thar FDM & Quattro Premier XE (MS/MS)
- Raw data says peak height 2000x higher with heated fixed restrictor

Heated fixed restrictor (full 4 ml/min flow into MS) dramatically improves SFC/MS performance relative to BPR split interface

MS detection: SFC & LC
for chiral bioanalysis

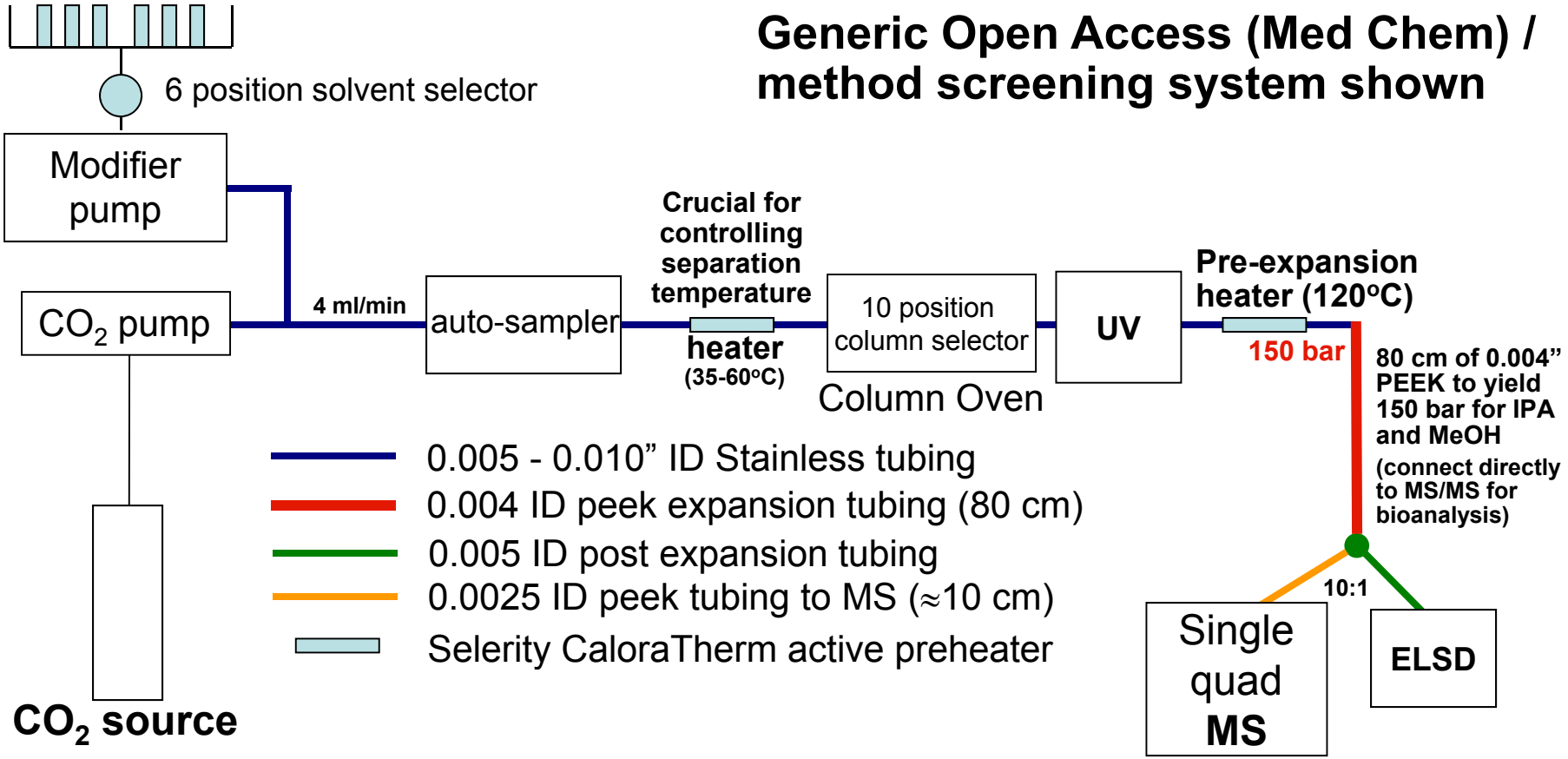


- 2 samples each from 44 animals + half of all samples were serial diluted 3 times to ensure no detector saturation for a total of 210 samples each run 4 times. LC and SFC used same MS/MS. Peak shape and response were steady throughout the 840 analyses. Concentrations vary from 40 to 90 ng/ml.
- SFC: S/N 3k with MS ≈ MS/MS
- LC: S/N 6k with MS/MS > MS (LC vs. SFC due to 100% MeOH vs. CO_2/IPA, i.e. viscosity)
- Animal to animal variation in enantiomer ratio <2%
- Brain to plasma (same animal) variation in enantiomer ratio ≤1%
- **SFC is clearly on par with RP-LC with respect to using MS detection**

Summary: comparison of both SFC with BPR / split & heated fixed restrictor detector interfaces with RP-LC

- **UV detection**
  - SFC: heated fixed restrictor provides lower noise (2x @ 150 bar) than BPR but column pressure seems to be most important
  - RP-LC: still has 5x lower noise and detection limits (needed for relatively few applications)
  - Is this a reason to take SFC column exit pressures to 200-300 bar? Maybe so for some applications where >10^3 dynamic range needed (another reason to go UHP-SFC)
  - Cooling the eluent stream and the UV detector appears to have real potential in further improving UV detection with SFC and reaching equivalence with LC
- **ELSD**
  - SFC: heated fixed restrictor clearly provides lower noise plus higher sensitivity and dynamic range than SFC with BPR or RP-LC
- **MS**
  - SFC: heated fixed restrictor provides much higher (ca. ≥10^2) sensitivity than BPR / split approach
  - SFC provides equivalent results to RP-LC at concentrations >100 pg/ml but still needs to be proven at lower concentrations

Our current "standard" in SFC detector interfacing



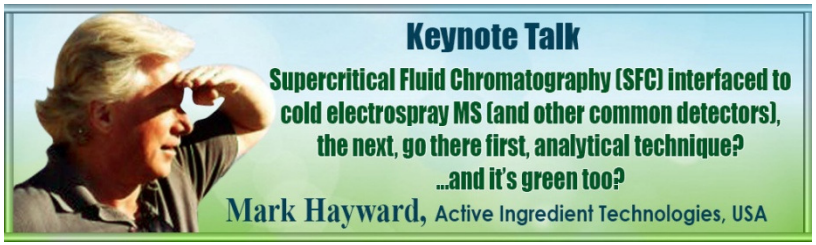
Generic Open Access (Med Chem) / method screening system shown

Suggested no BPR needed:
J.D. Pinkston, *Eur.J. Mass Spectrom.* 11, **2005**, 189.
Full flow into heated APPI, ESI, APCI MS sources with good results:
R.A. Coe, J.O. Rathe, J.W. Lee *J. Pharm. Biomed. Anal.* 42, **2006**, 573.
Noted importance of temperature for MS interface:
F Sadoun, H. Virlizer, P.J. Arpino *J. Chromatogr.* 647, **1997**, 351.

For high sensitivity bioanalysis, eliminate UV / ELSD and replace MS with triple quad MS/MS (full flow from restrictor into MS source)

Other SFC applications

Focus on productivity



Supercritical Fluid Chromatography (SFC), the next go there first technique!

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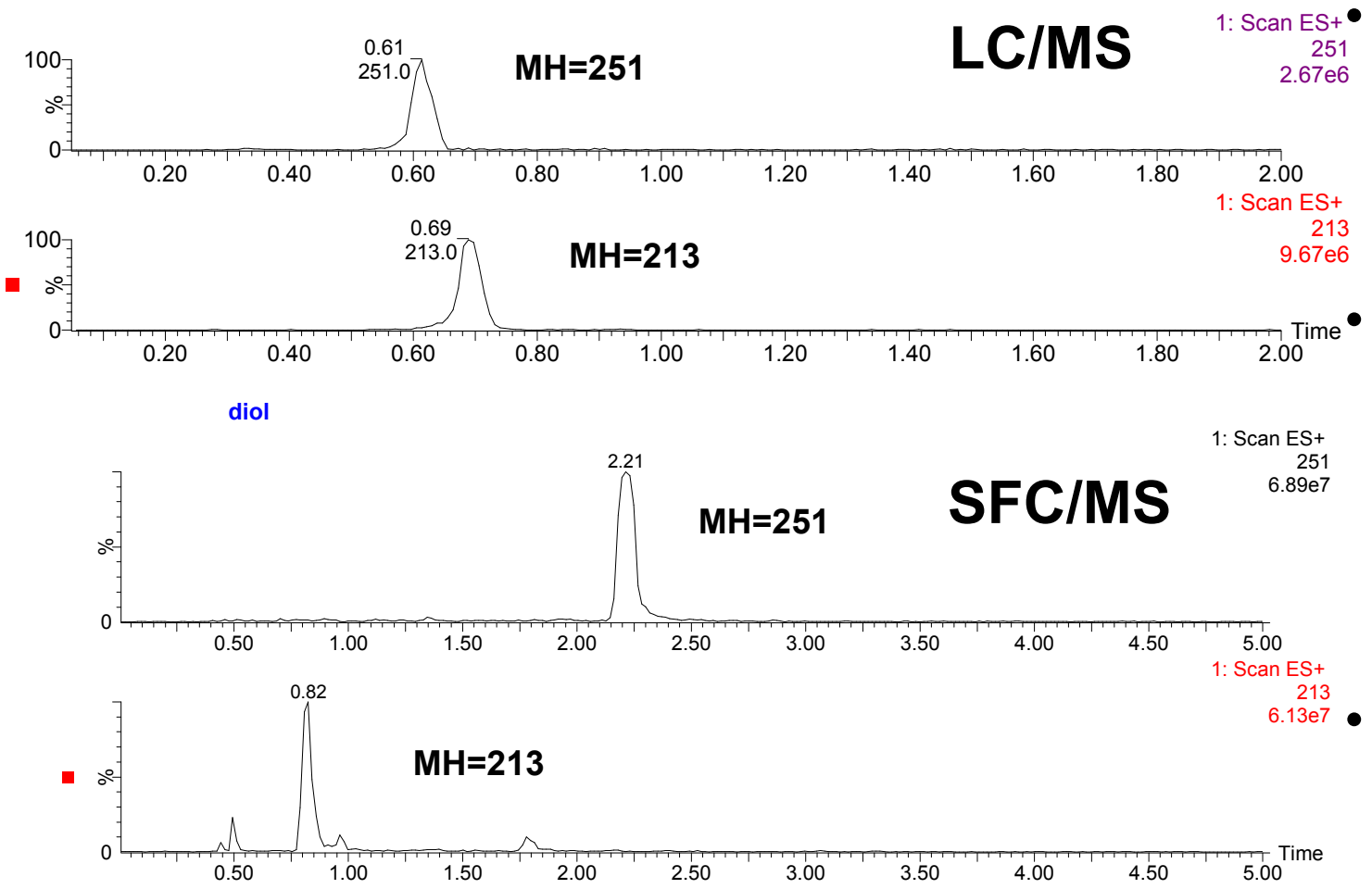
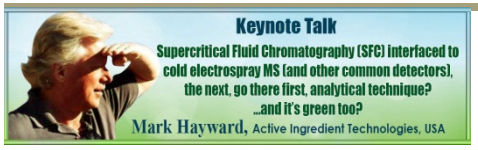
Highlight of the application of SFC: Open Access (OA) SFC/UV/ELSD/MS

- To gain efficiency, complementary capabilities, and greater capacity, we have deployed OA-SFC/UV/ELSD/MS
  - True orthogonal separation option for Med Chem support (TLC with awesome detectors)
  - Still has broad overlap with RP-LC/UV/ELSD/MS for Med Chem support, thereby providing added capacity for routine reaction monitoring
  - Also opens up chiral method development and ee measurement to “everyone”
  - 3 achiral column choices & 7 for chiral (6 modifier / buffer options)
- Using the detector interfacing techniques described herein and recent software releases, SFC/UV/ELSD/MS is ready for prime time in providing immediate gratification in the above applications

Orthogonal SFC separations can be highly complementary to the frequently used RP-LC



- Truly orthogonal SFC approach can separate starting material and products that RP-LC can't
- These SFC methods also are aligned with preparative scale methods allowing immediate purification
- MS used in this application due to lack of chromaphore



Normal phase separation gives TLC-like outcome for polar intermediates

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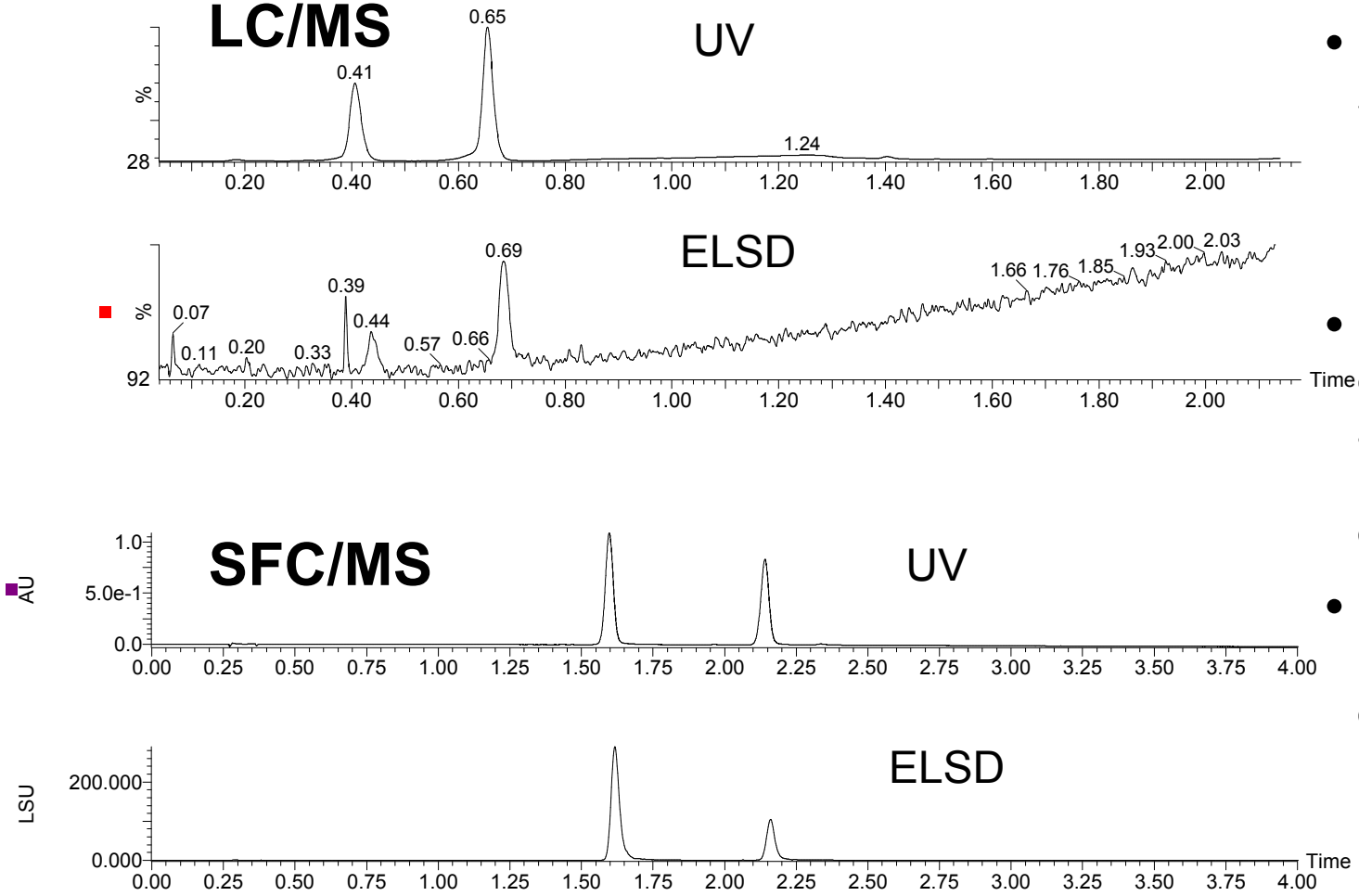
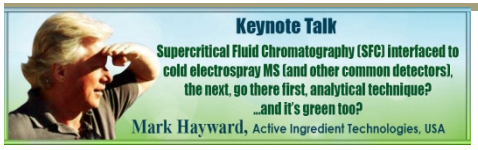
OA-SFC/UV/ELSD/MS can provide similar information as OA-LC/UV/ELSD/MS



- Chromatograms showing starting material and product (reaction progress)
- Essentially same data with either approach except reverse elution order (TLC-like)
- Note the improved quality of ELSD with SFC!



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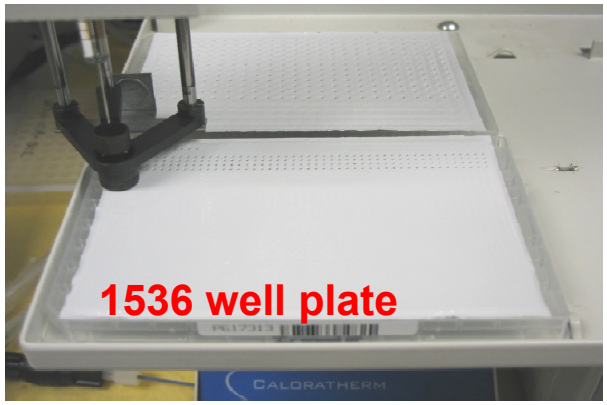
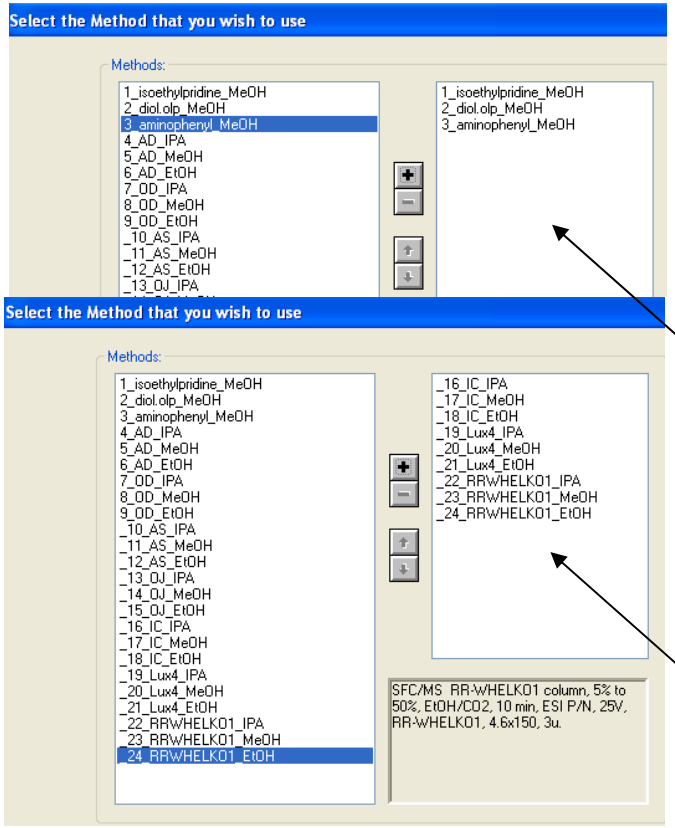
A systematic evaluation of SFC applicability compared with RP-LC



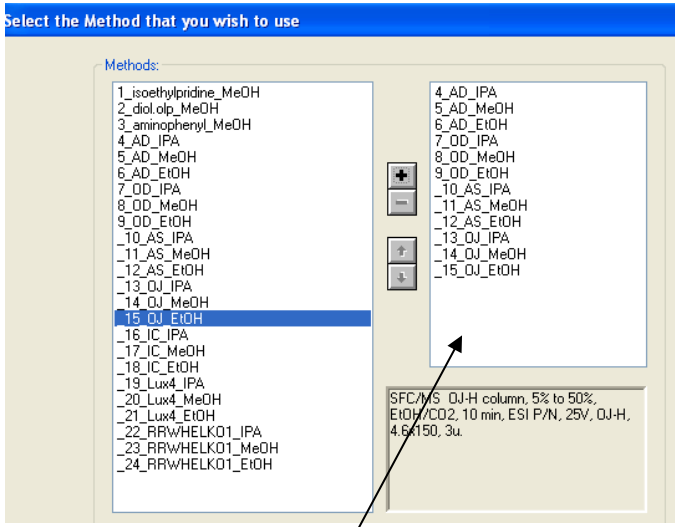
- 1536 diverse CNS drug like compounds (SBD screening set, 80-98% purity, LogP 1-5)
- Each compound measured by generic (grad) RP-LC/UV/ELSD/MS and SFC/UV/ELSD/MS
- 96% gave completely equivalent results
  - Good separation / peak shapes (rev elution order) with all 3 detectors (UV/ELSD/MS) and agreement on purity
  - 3% SFC gave better separation (bias toward more polar compounds)
- **Conclusion: There is huge overlap in the applicability of SFC and RP-LC**
- As a result, we have diverted normally LC projects to SFC in order to meet capacity and timeline needs and achieved consistent project success

Chiral screening of many methods on a single sample login (MassLynx / OpenLynx SCN 798)



Method set for achiral analysis

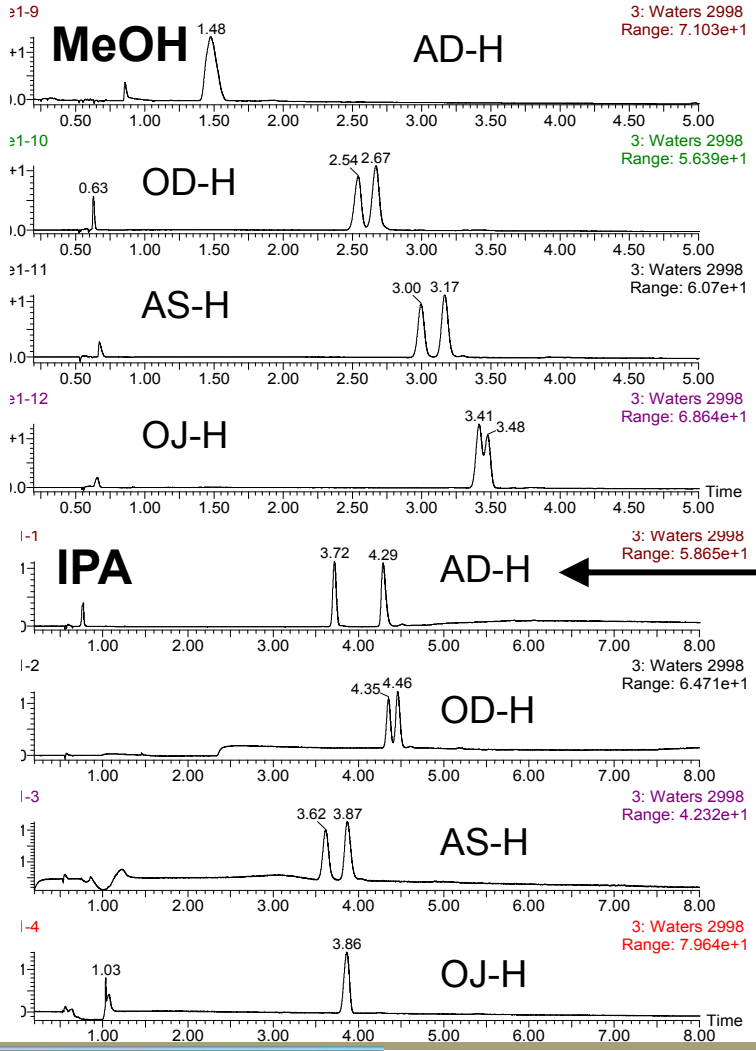
Second set of methods for chiral column screening if first set don't work

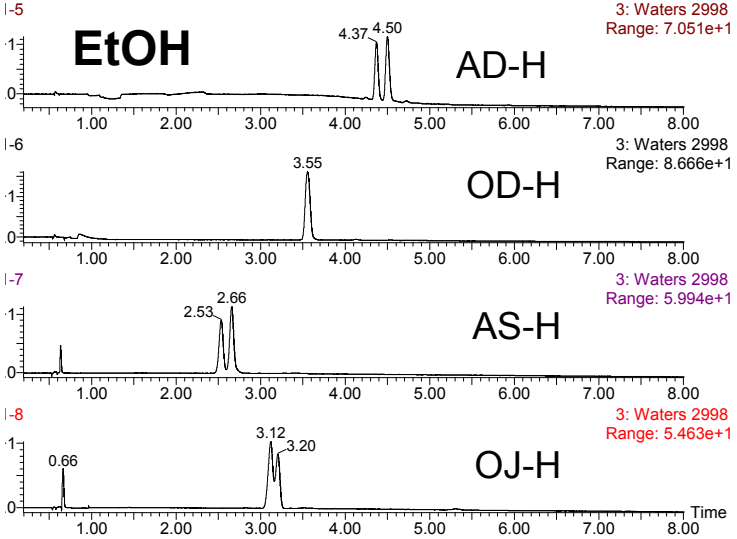


First set of methods for chiral column screening

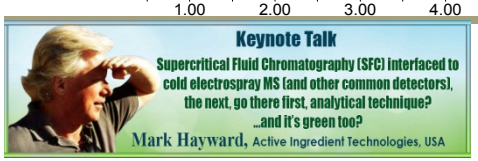
Software makes method screening easy for complex separations!

Screening chiral conditions for preparative method development (4 x 3)





- **Screening 4 columns and 3 solvent gradients showed AD-H with IPA gives a useful separation**
- **Scaled preparative version of same method was immediately used to resolve 10g on same day**
- **OA-SFC/UV/ELSD/MS is a viable screening approach for preparative work**
- **Seemingly complex made simple?**



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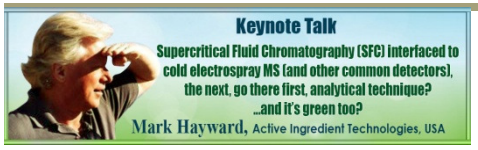
Conclusions:

- SFC is capable of highly complex separations (chiral) and at the same time is broadly applicable much like RP-LC
- **IT is the next, go there first, analytical technique!**

- **…and it's green too? Absolutely!***
- For SFC, 75% of solvent is CO_2 (taken from and returned to air)
  - 1/4 volume = 1/4 waste disposal costs
- Remaining 25% solvent is alcohol (friendliest)
  - Both alcohol and CO_2 (bulk) cost less than ACN (RP-LC)



*Marek Tobiszewski, Jacek Namiesnik
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