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Evaluation of Flash SFC for Pharmaceutical Medicinal Chemistry Purifications

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Outline

- Introduction to Medicinal Chemistry Open Access Purification In Amgen
- Correlation of TLC to SFC
- Evaluation of injection techniques for flash SFC
- Evaluation of loading under flash SFC conditions
- Conclusions

SFC and Pharma Discovery Purification

- **Specialized Purifications (dedicated function)**
  - **Chiral: Majority of purifications utilize SFC**
  - **Achiral (singleton): SFC use increasing but LC still major workhorse**
  - **Library: Majority utilize LC, use of SFC increasing**

- **Open Access (performed by medicinal chemist)**
  - **High performance separations: mainly utilize reverse phase HPLC**
  - **Low performance separations: mainly performed using flash chromatography (50 μ silica gel, organic solvents)**
  - **Currently SFC has made limited inroads into this purification arena**

Flash chromatography

- **Purification workhorse in medicinal chemistry laboratories at Amgen**
  - **One flash system per 1-2 chemists**
  - **Used by chemists 5-15 times/week**
- **What is flash chromatography?**
  - **Normal phase (silica gel)**
    - **Prepacked cartridges, 40-60 micron, 4-200 g (larger size available)**
    - **Scale up from TLC plates**
  - **High loading (loading of 25-100:1 silica:sample)**
    - **2 x 25 cm column; 470 mg – 1.88 g**
  - **Organic solvents**
    - **DCM/methanol/ammonium hydroxide gradients**
    - **Ethyl acetate/hexane gradients**
  - **Injection**
    - **Via syringe, pump, or solid injection (insoluble samples)**

Flash Chromatography

- **Advantages**
  - Automated
  - Reliable
  - Easy to use
  - Technique learned in grad school
  - Easy scale up from TLC
  - Disposable cartridges
    - Limits cross contamination
  - Low pressure (< 100 psi)
  - Organic solvents easy to remove
    - Volatile modifiers
- **Disadvantages**
  - Low efficiency due to larger particles
  - Large amounts of solvent used
  - High consumable cost

Green Flash

- **Can SFC be utilized for flash purifications?**
- **Advantages**
  - **Reduced solvent consumption**
  - **Lower pressure drop = smaller particles = higher efficiency**
  - **Easier product recovery**
- **Disadvantages**
  - **New technology to learn**
  - **CO_2 supply**
  - **Fraction collection**

Requirements for success (my thoughts)

- Equipment (and approach to purification) as close as possible to existing flash technology used by medicinal chemistry
- Cost no more than 2x existing flash equipment
- Easily interchanged cartridges
  - Are disposable cartridges cost effective w/ small particles?
- Open bed fraction collection (no cyclone separators)
  - Will this limit maximum flow?; won't take advantage of high linear velocities possible with SFC
- Ability to scale from TLC plates
  - Eliminate need for analytical SFC analysis prior to purification
- Scale to allow use of CO_2 cylinder (no BDS/GDS)

Previous Work

- **Thar proof of concept**
  - **1.3 g acetaminopen, 2.2 g benzoic acid separated using methanol/CO2 on silica cartridge**
    - **Good purities, peak shape observed**
- **Mike Burns, Modular SFC**
  - **Poster @ SFC 2009**
  - **One example**
    - **Scale from CN HPTLC (5 μ) to CN prep column (6 μ)**
    - **Solid injection**
    - **Good correlation TLC to SFC**
    - **Issues?**
      - **Used 5 um particles**
      - **No loading experiments**

Questions to be addressed

- **Can TLC be used to scale up to SFC?**
- **What is best injection technique for flash SFC?**
- **Can loadings seen with Flash LC be achieved with Flash SFC?**
- **What is impact of smaller particle silica gel on Flash SFC loading?**

Experimental

- **77 commercially available drug like compounds**
  - **Mix of neutral, basic and acidic compounds**
- **TLC Methods**
  - **5/95 methanol/dichloromethane**
  - **5/95/0.5 methanol/dichloromethane/NH_4OH**
  - **25/75 ethyl acetate/heptane**
  - **100 ethyl acetate**
  - **99/1 ethyl acetate/NH_4OH**
  - **- 10/90 methanol/dichloromethane**
  - **- 10/90/1 methanol/dichloromethane/NH_4OH**
  - **- 50/50 ethyl acetate/heptane**
  - **- 50/50/0.5 ethyl acetate/heptane/NH_4OH**
- **SFC Methods**
  - **15 micron silica gel (4.6 x 150 mm)**
  - **5-55% gradient over 8 minutes**
  - **5 ml/min, 100 bar**
  - **Co-solvents**
    - **Methanol w/ 0.2% diethylamine**
    - **50/50 methanol/dichloromethane with 0.2% diethylamine**
    - **50/50 methanol/ ethyl acetate with 0.2% diethylamine**
    - **Methanol**
    - **50/50 methanol/dichloromethane**
    - **50/50 methanol/ethyl acetate**

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TLC to SFC Correlation

- **Can TLC be used to assess separation prior to Flash SFC?**

	SFC co-solvent					
TLC Conditions	MeOH/ DEA	MeOH/ DCM/DEA	MeOH/ EA/DEA	MeOH	MeOH/ DCM	MeOH/ EA
5/95 MeOH/DCM	0.53	0.52	0.51	0.39	0.37	0.31
10/90 MeOH/DCM	0.52	0.49	0.55	0.39	0.37	0.31
5/95/0.5 MeOH/DCM/NH_4OH	0.40	0.39	0.51			
10/90/1 MeOH/DCM/NH_4OH	0.07	0.11	0.19			
25/75 EA/heptane	0.40	0.34	0.40			
50/50 EA/heptane	0.51	0.47	047			
100 EA	0.30	0.26	0.20			
50/50/0.5 EA/heptane/NH_4OH	0.41	0.38	0.46			
99/1 EA/NH4OH	0.43	0.41	0.48			

TLC to SFC Correlation

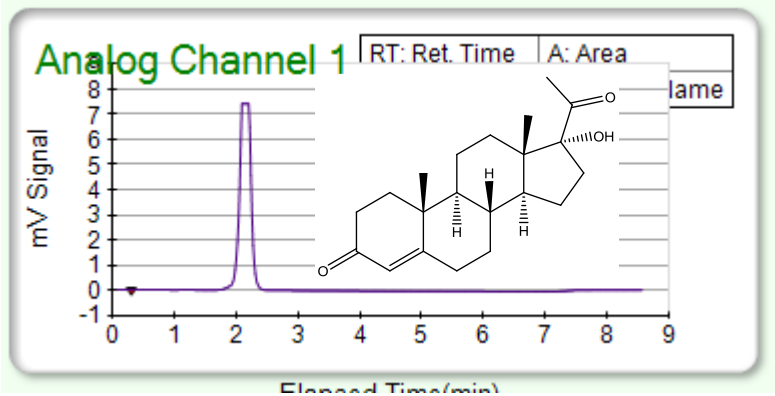
- **Correlation not strong for TLC R_f to SFC R_t**
- **Poorer correlation for more polar TLC systems (10/90/1 MeOH/DCM/NH_4OH and 100 EA) due to large number of samples with R_f >0.8**

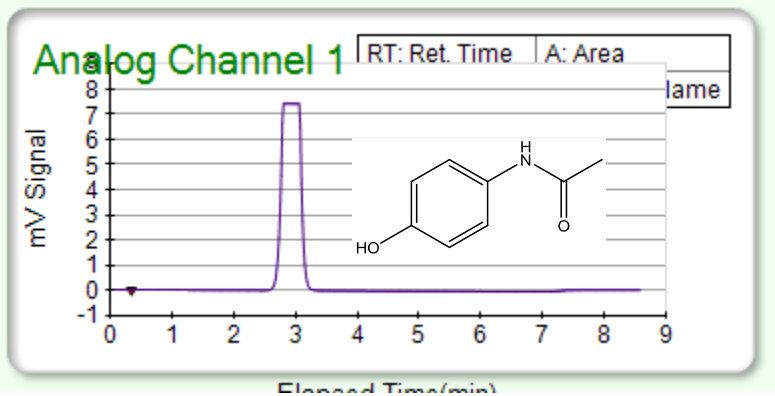
Compounds	TLC α *	SFC α **
Hydroxyprogesterone, Acetamidophenol	3.85	1.04
Tefrenadine, Propafenone hydrochloride	2.90	1.02
Acetaminophen, Carbamazepine	5.3	1.01
Indapamide, Flurbiprofen	1.04	1.38
Promethazine, Guaifenesin	1.04	1.70

* 10/90 methanol/DCM
** methanol/DEA co-solvent

Is loading comparable between flash SFC and LC?

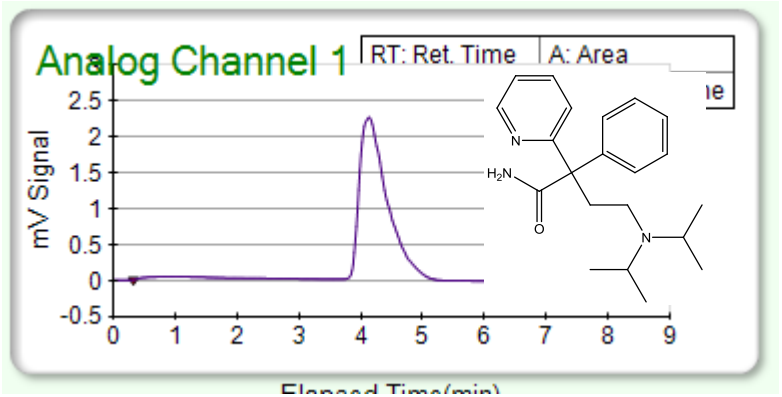
- **Experimental**
  - 19 x 150 mm column (~ 30 g packing)
    - 15, 30 and 50 μ silica gel
  - 5-55% methanol/DEA gradient
  - 80 ml/min, 100 bar BPR
  - 28 compounds injected @ 50 mg load (1-2 ml injections)
  - Modifier stream injection
  - Neutral Compounds (hydroxyprogesterone, acetaminophen)

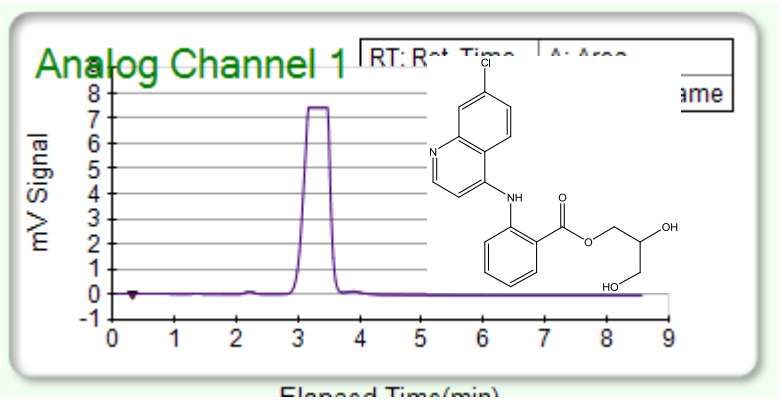




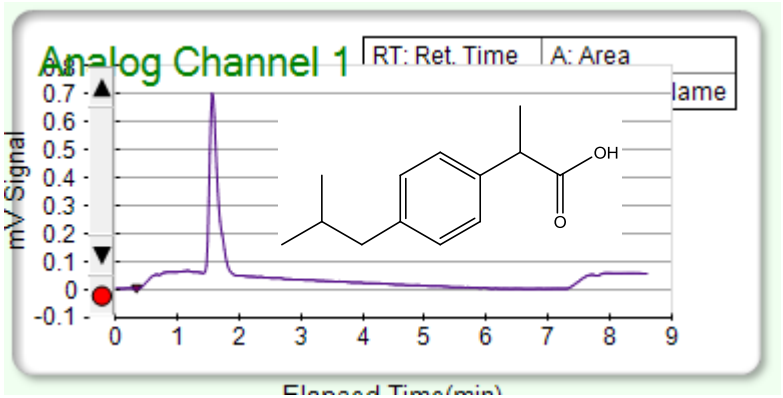
Test Injections (50 mg injections)

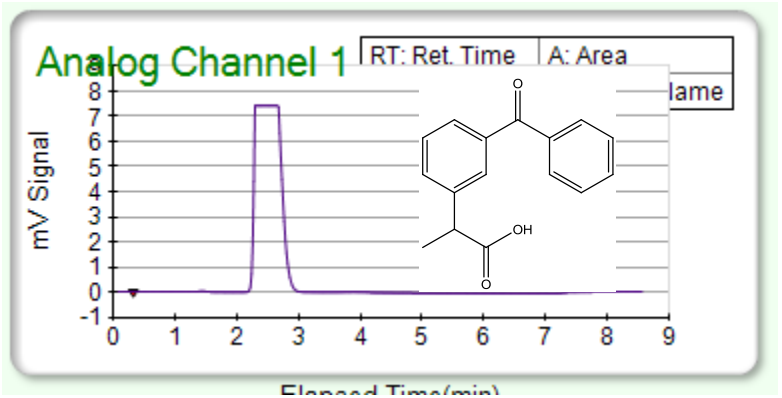
- **Basic Compounds (disopyramide, glafenine)**





- **Acidic Compounds (ibuprofen, ketoprofen)**



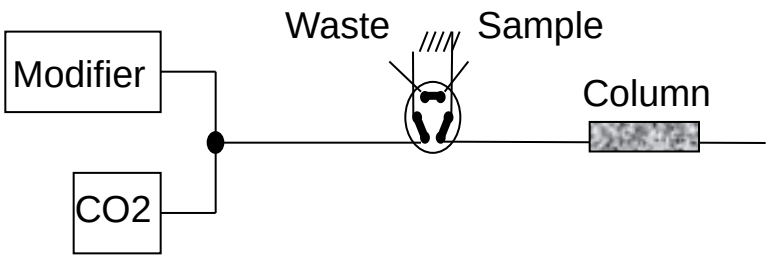


Preparative Loading Studies

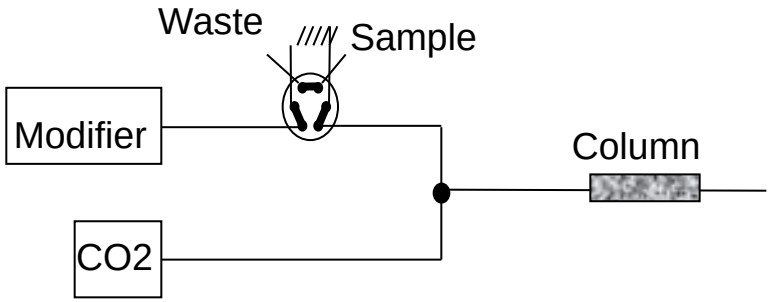
- 19 x 150 mm 50 μ column, modifier stream injection
- Loads: 100-500 mg
- Results:
  - Acetaminophen: @ 300 mg pressure shutdown
  - Carbamazepine: @ 400 mg pressure shutdown
  - Tetracaine: @ 400 mg pressure shutdown
- Is compound coming out of solution due to low methanol level (5%) at start of gradient?
- Evaluated acetaminophen under isocratic (20% methanol/DEA) conditions
  - Able to inject 500 mg without pressure shutdown

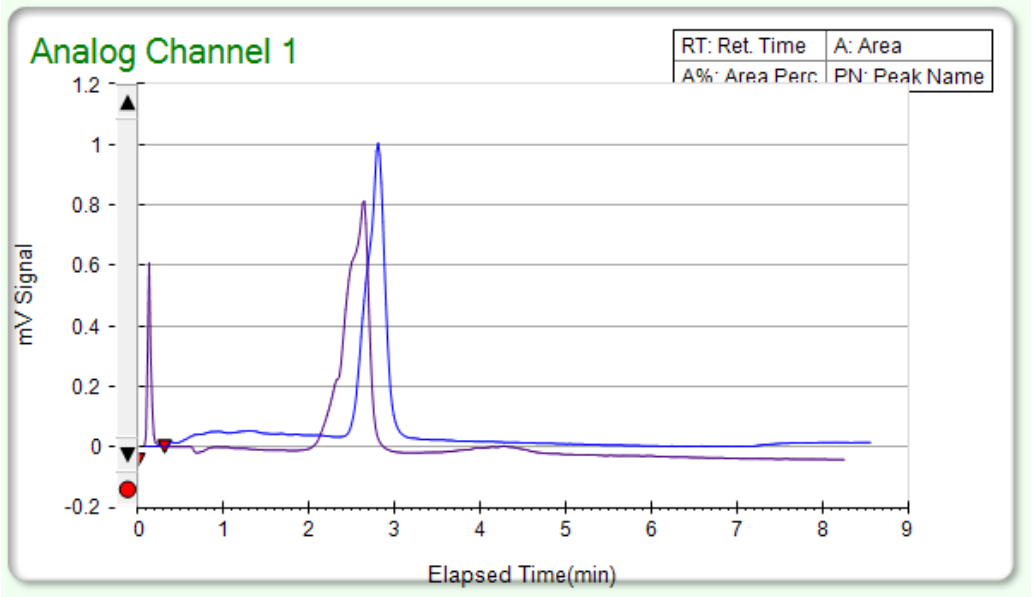
Mixed Stream vs. Modifier Stream Injection

Mixed stream Injection

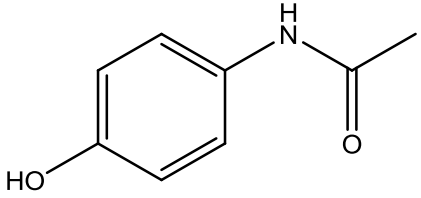


Modifier stream injection™





- **200 mg (2 ml in DCM/MeOH) acetaminophen on column**
- **Blue: modifier stream injection Purple: mixed stream injection**

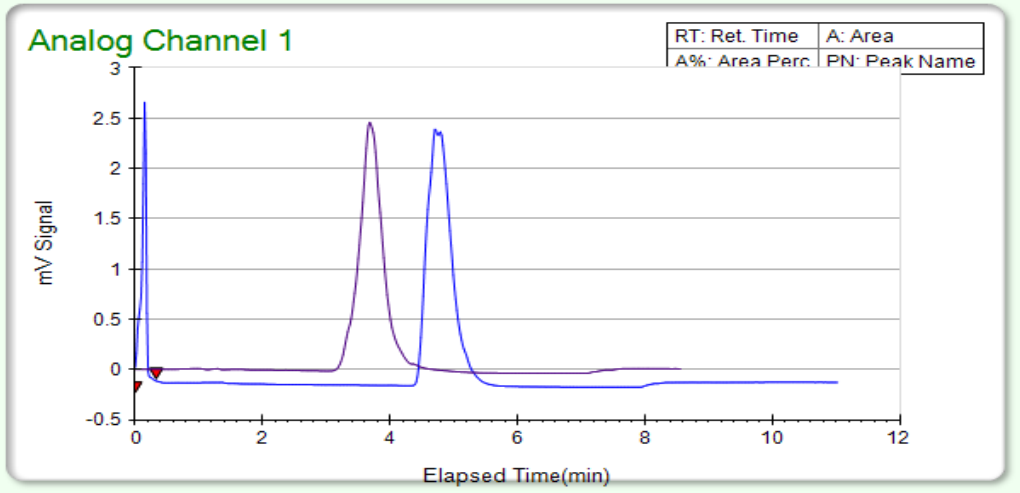


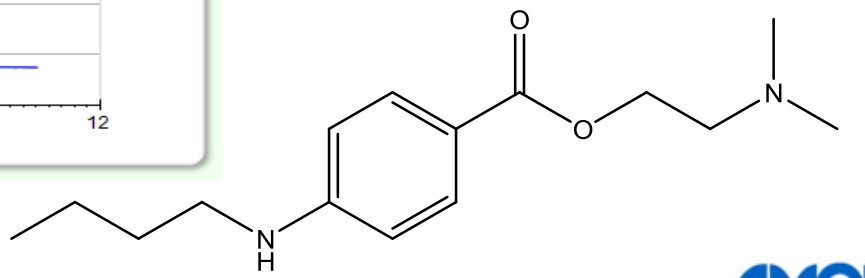
Evaluation of “dry loading” injection technique

- Dry loading often used in flash LC for injection
  - Allows purification of poorly soluble samples
  - Eliminates effect of large injection volumes for samples with lower solubility
- Also known as: impregnation, solid injection, dry pack
- Process: dissolve sample, add silica, remove solvent under vacuum, pack in pre-column, purify sample
- Will same process be advantageous with flash SFC?

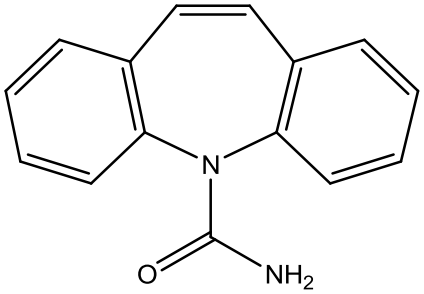
Purification of Tetracaine HCl with dry load

- Modifier stream injection: @ 400 mg pressure shutdown
- Dry Load: Able to injection 500 mg without issues
- 200 mg on-column (Purple = modifier stream, blue = dry load)

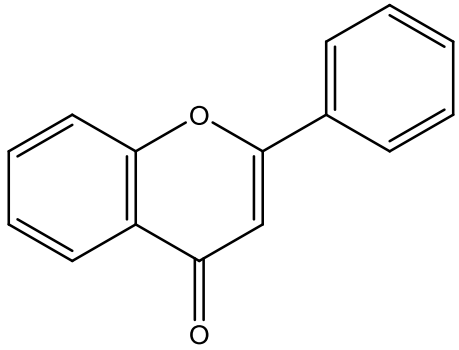




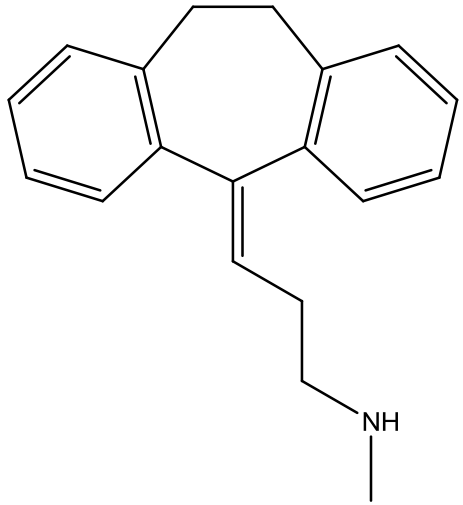
Test Compounds



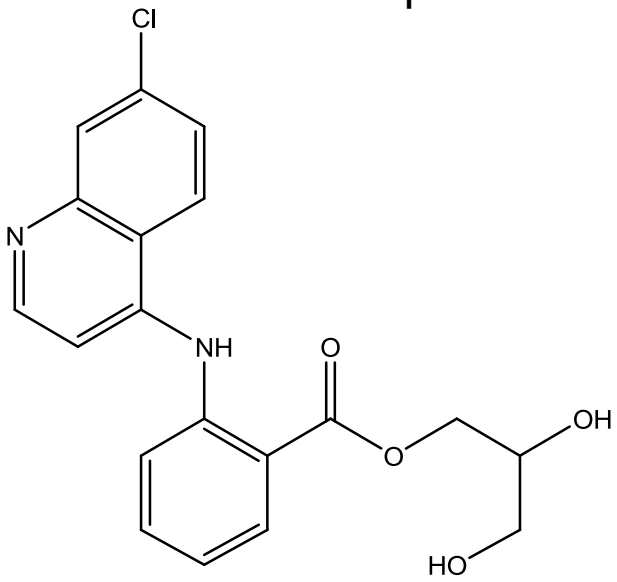
Carbamazepine



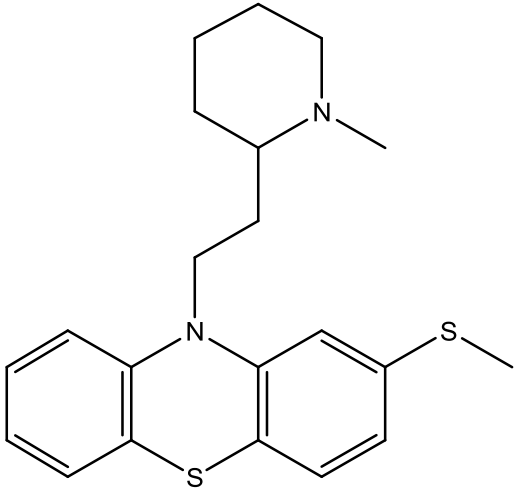
Flavone



Nortriptyline



Glafenine



Thioridazine

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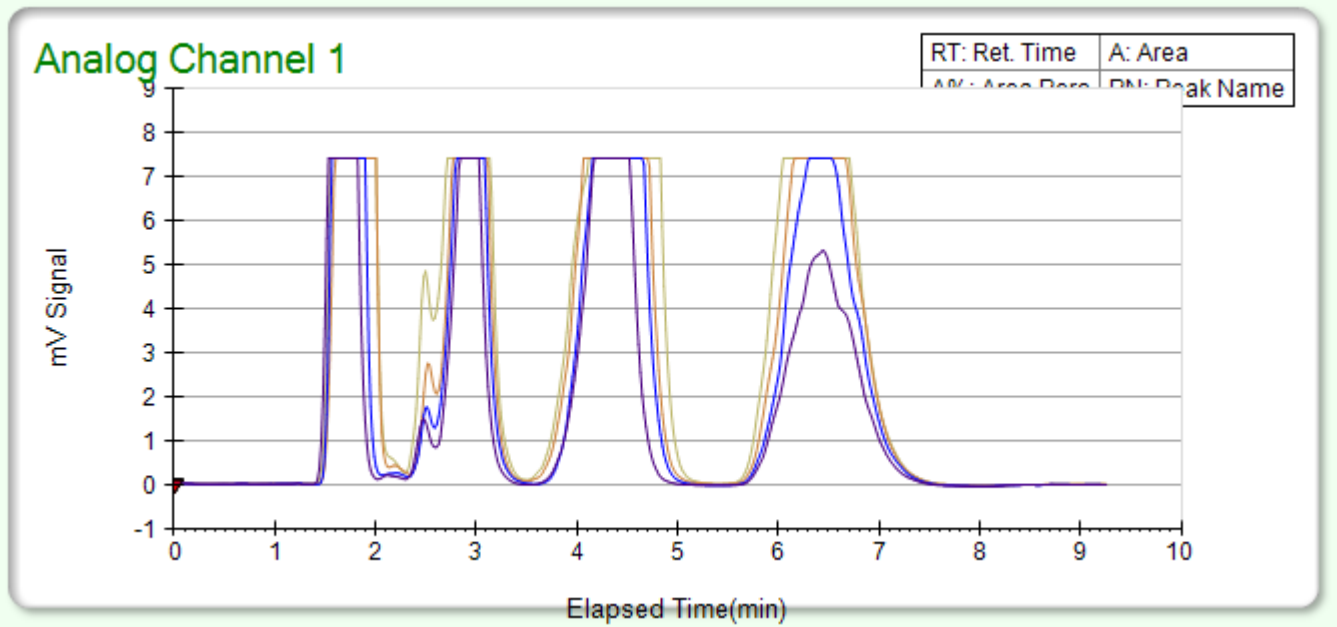
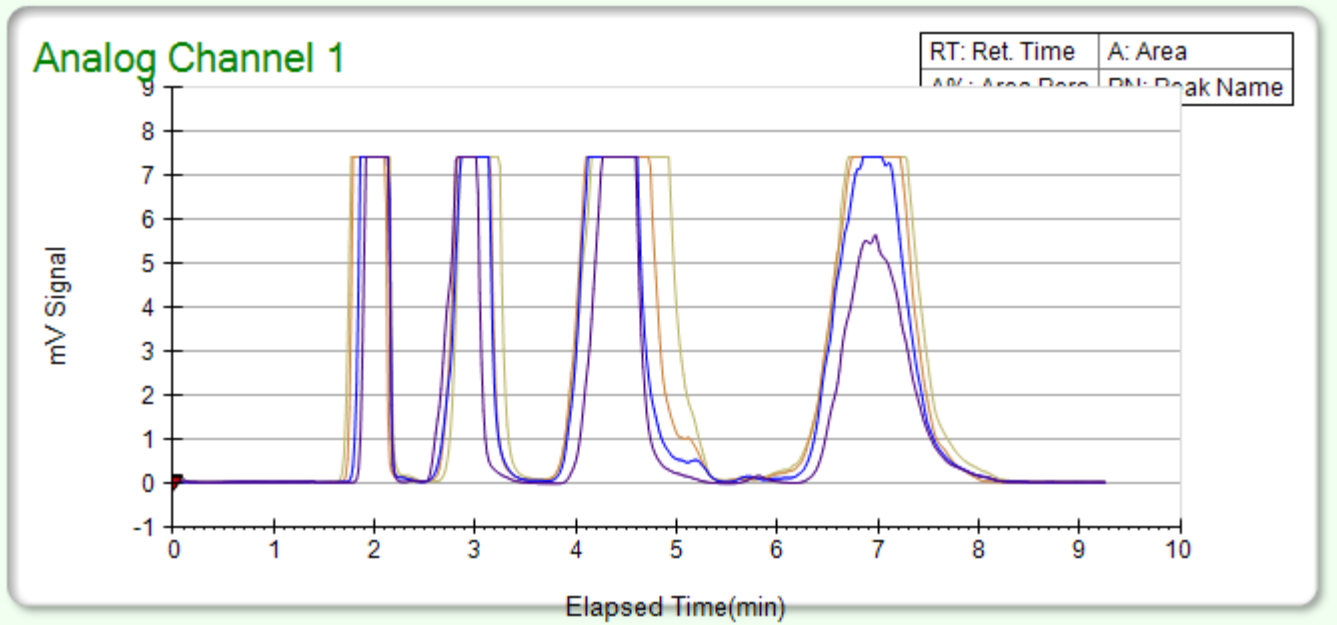
Loading studies using dry load

- Carbamezepine, flavone, glafenine, thioridazine
- Sample mixture prepared 10:1 (w/w) with silica
- 200-500 mg on 50 micron column



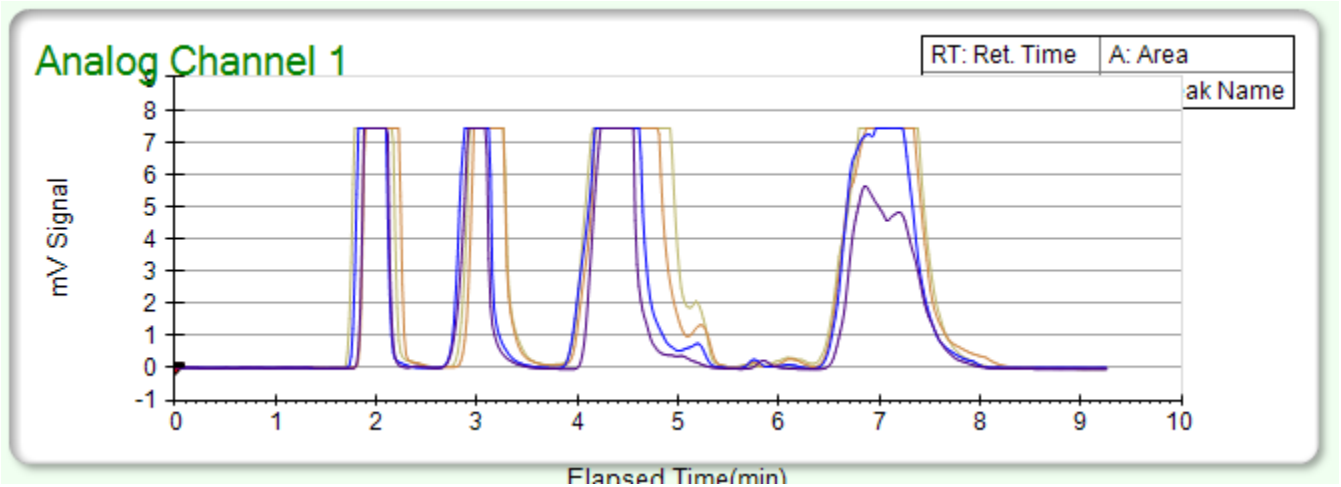
Evaluation of 30 micron column

- **100-500 mg on column**

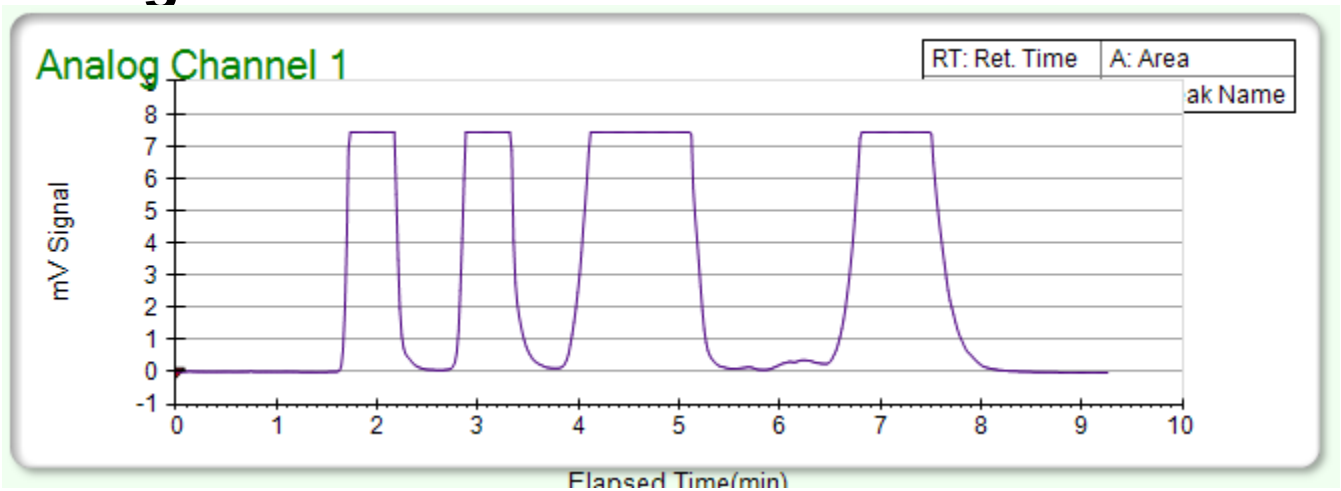


Evaluation of 15 micron column

- **200-500 mg on column**

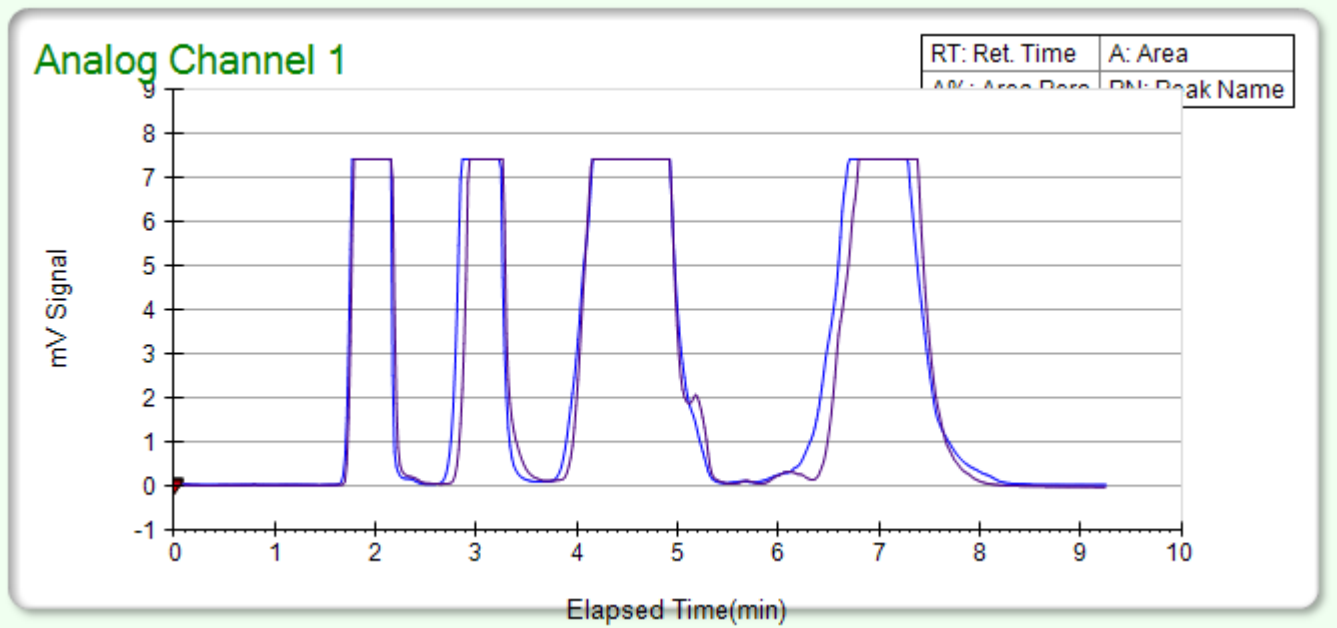


- **780 mg on column**



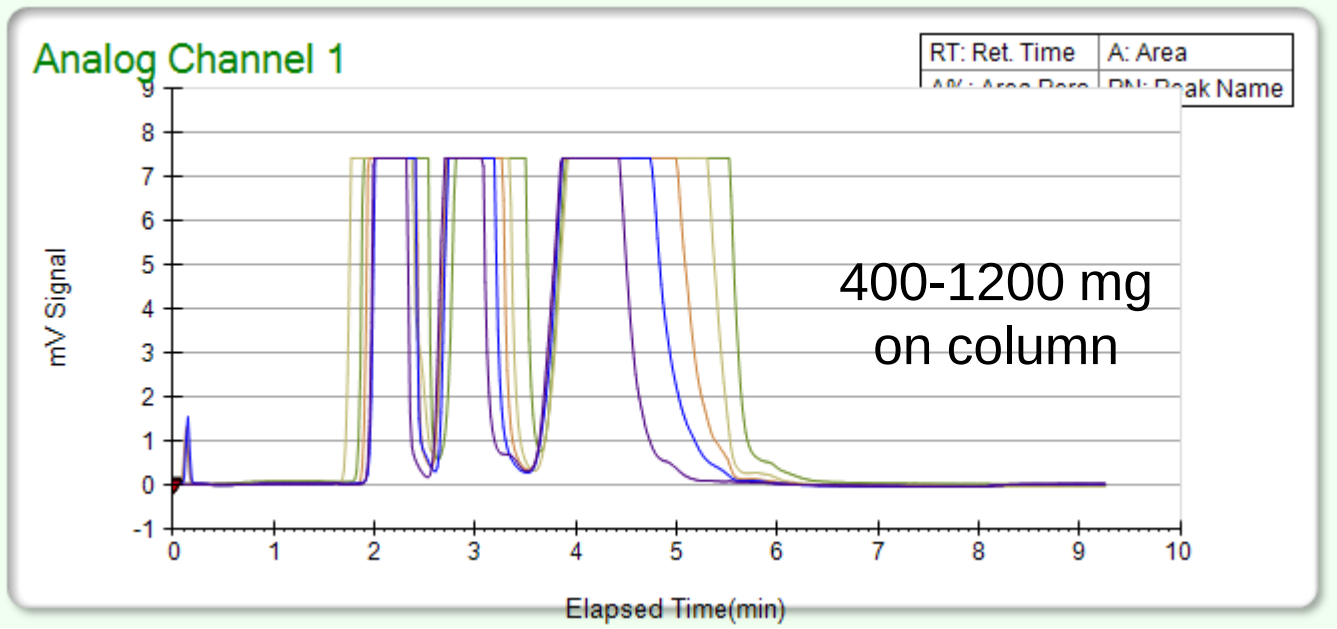
15 vs. 30 micron

- **Purple = 15 micron, Blue = 30 micron**



Mixture 1: SFC Separation

- **Carbamezepine, flavone, glafenine**
- **Sample mixture prepared 10:1* (w/w) with silica**
- **400-2000 mg on 30 micron column**

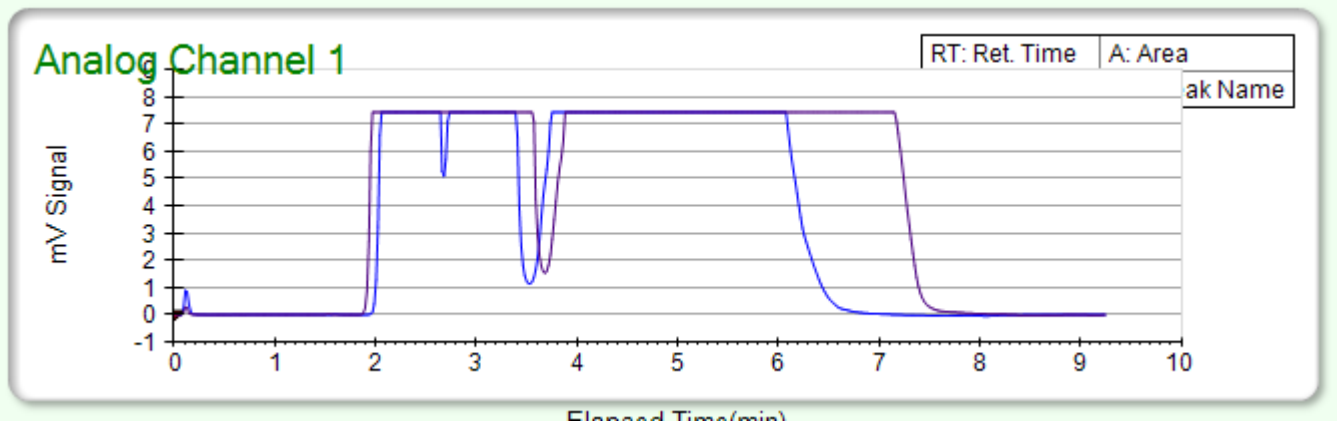


Purities
Peak 1: 100%
Peak 2: 99.6%
Peak 3: 98.4%

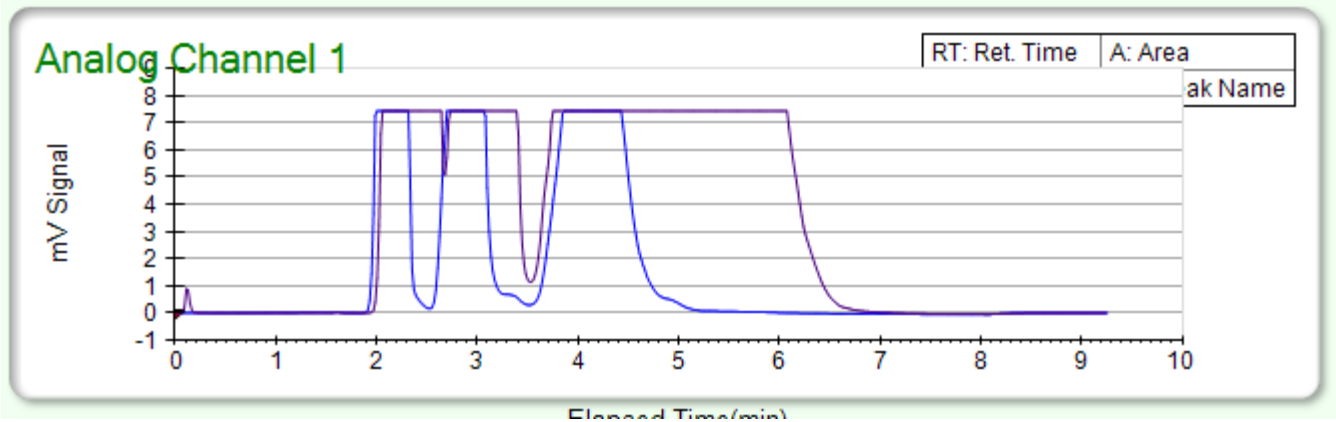
* Loads greater than 1.2 g used 5:1 ratio

Mixture 1 SFC Separation (cont.)

- **1600 and 2000 mg on column**



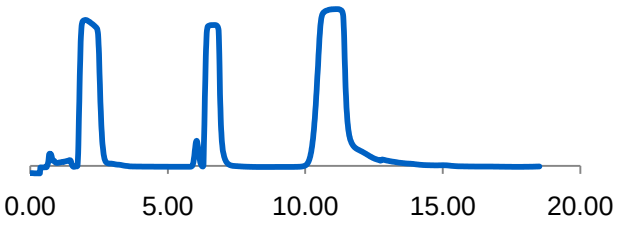
- **400 and 1600 mg on column**



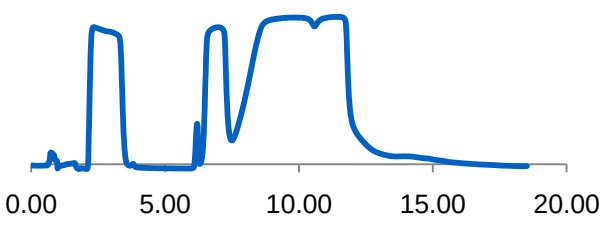
Mixture 1: LC Separation

- **Flash LC Conditions: methanol/DCM/NH4OH gradient (0.5/99.5/0.05 to 10/90/1) over 18.5 minutes**
- **19 x 150 mm 30 micron column @ 40 ml/min**
- **400 and 600 mg injections**

400 mg Mixture 1



600 mg Mixture 1



- **Pressure shutdown @ 800 mg**
  - **Peak 3 (glafenine) has low DCM solubility (< 5 mg/ml)**

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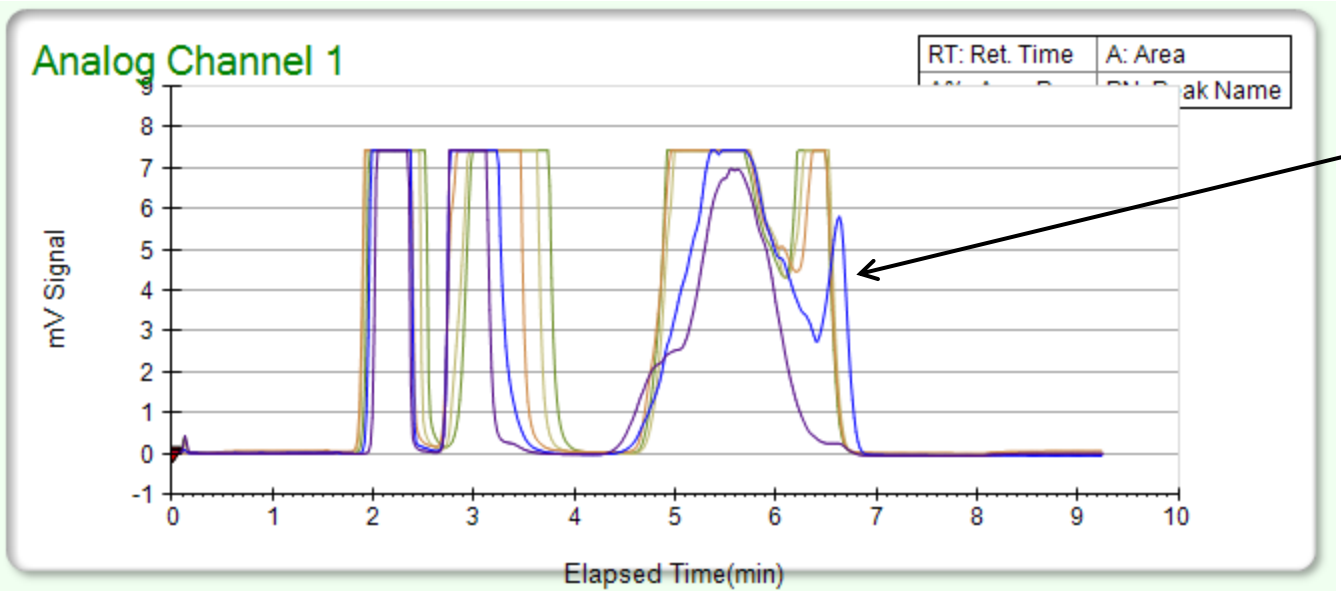
Mixture 1: Flash LC/SFC Comparison

Technique	Load (mg)	Peak 1 Volume	Peak 2 Volume	Peak 3 Volume	Solvent (L/g crude)	Productivity (g/hr)*
LC	400	40	32	60	1.85	0.84
LC	600	56	45	200	1.23	1.26
SFC	400	4.2	9.0	22	0.56	1.68
SFC	600	5.0	9.8	37	0.36	2.52
SFC	1200	7.3	14.6	57	0.18	5.04

* Assume 10 minute re-equilibration for LC, 5 minutes for SFC

Mixture 2: SFC Separation

- **Carbamezepine, flavone, nortriptyline HCl**
- **Sample mixture prepared 10:1 (w/w) with silica**
- **400-1200 mg on 30 micron column (SFC)**

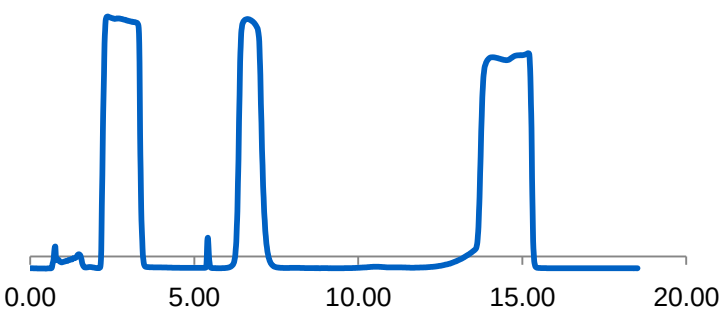


HCl salt: cause of poor peak shape?

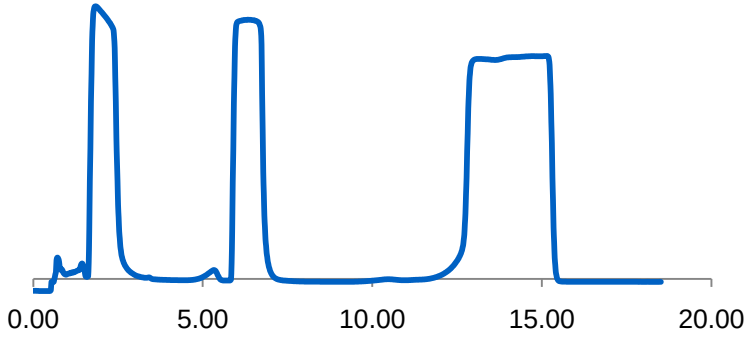
Mixture 2: LC Separation

- **400, 800, 1200 mg injections**

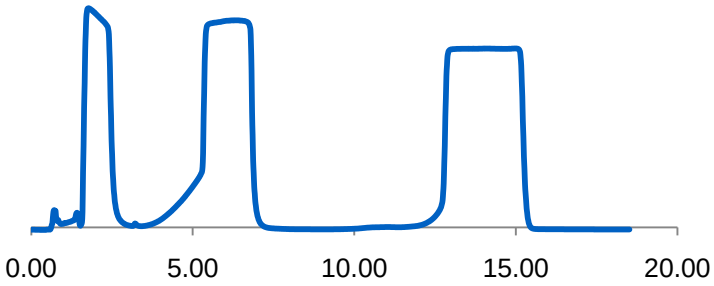
400 mg Mixture 2



800 mg Mixture 2



1200 mg Mixture 2



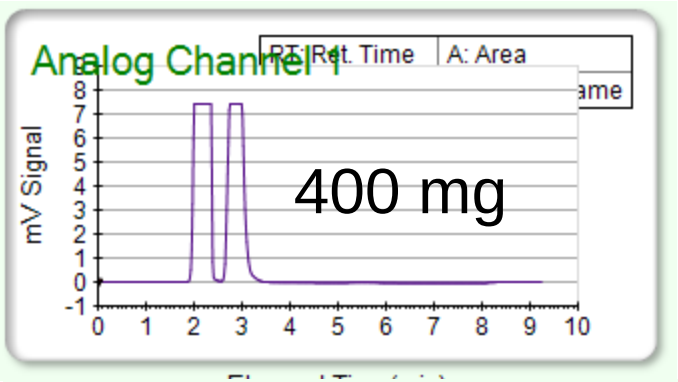
Mixture 2: Flash LC/SFC Comparison

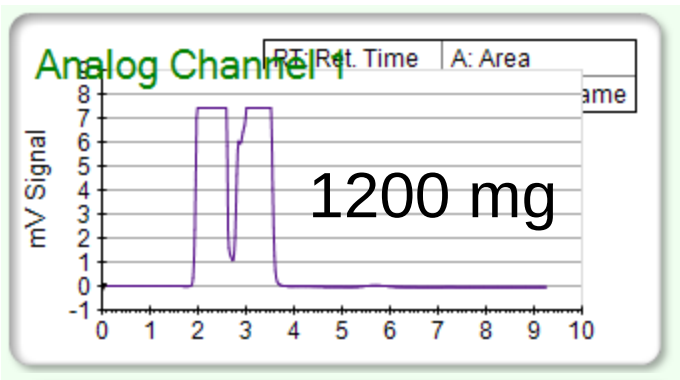
Technique	Load (mg)	Peak 1 Volume	Peak 2 Volume	Peak 3 Volume	Solvent (L/g crude)	Productivity (g/hr)*
LC	400	52	41	71	1.85	0.84
LC	800	38	43	110	0.93	1.68
LC	1200	43	100	108	0.62	2.52
SFC	400	3.9	7.6	48	0.56	1.68
SFC	800	5.7	14	60	0.28	3.36
SFC	1200	7.6	18	58	0.19	5.04

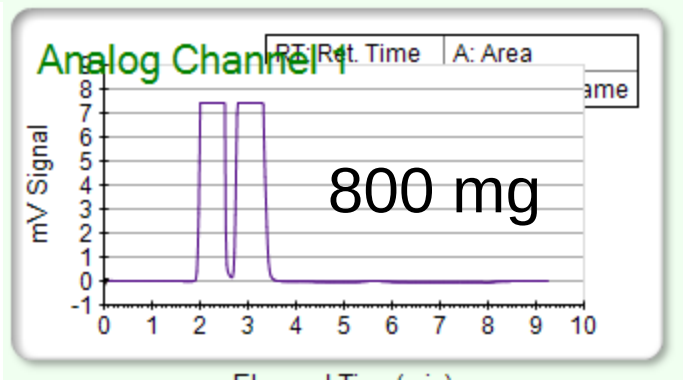
* Assume 10 minute re-equilibration for LC, 5 minutes for SFC

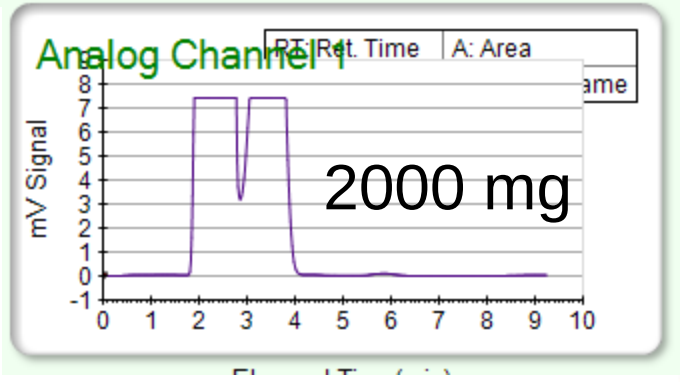
Mixture 3: SFC Separation

- **Carbamezepine, flavone**
- **Sample mixture prepared 5:1 (w/w) with silica**
- **400-2000 mg on 30 micron column (SFC)**





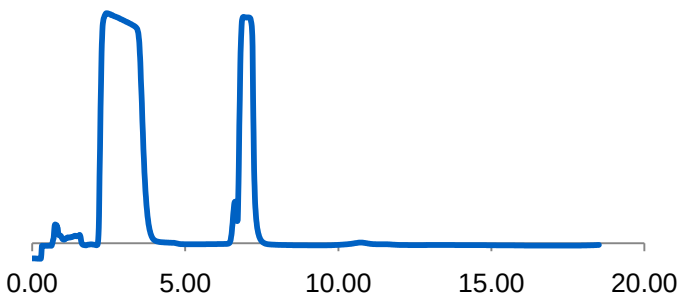




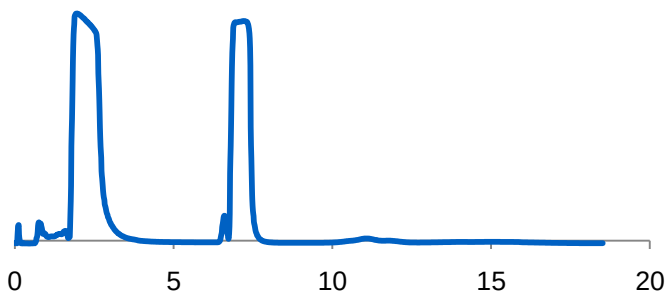
Mixture 3: LC Separation

- 400, 800, 1200 and 2000 mg injections

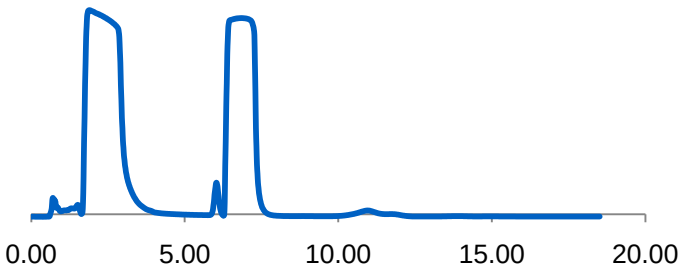
400 mg Mixture 3



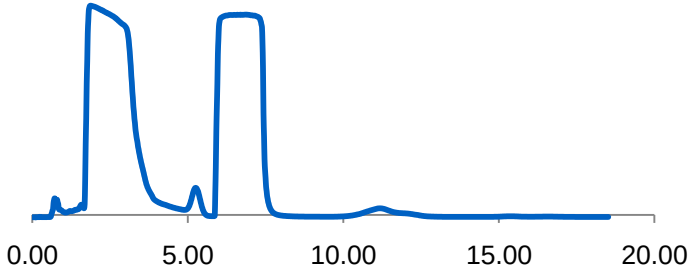
800 mg Mixture 3



1200 mg Mixture 3



2000 mg Mixture 3

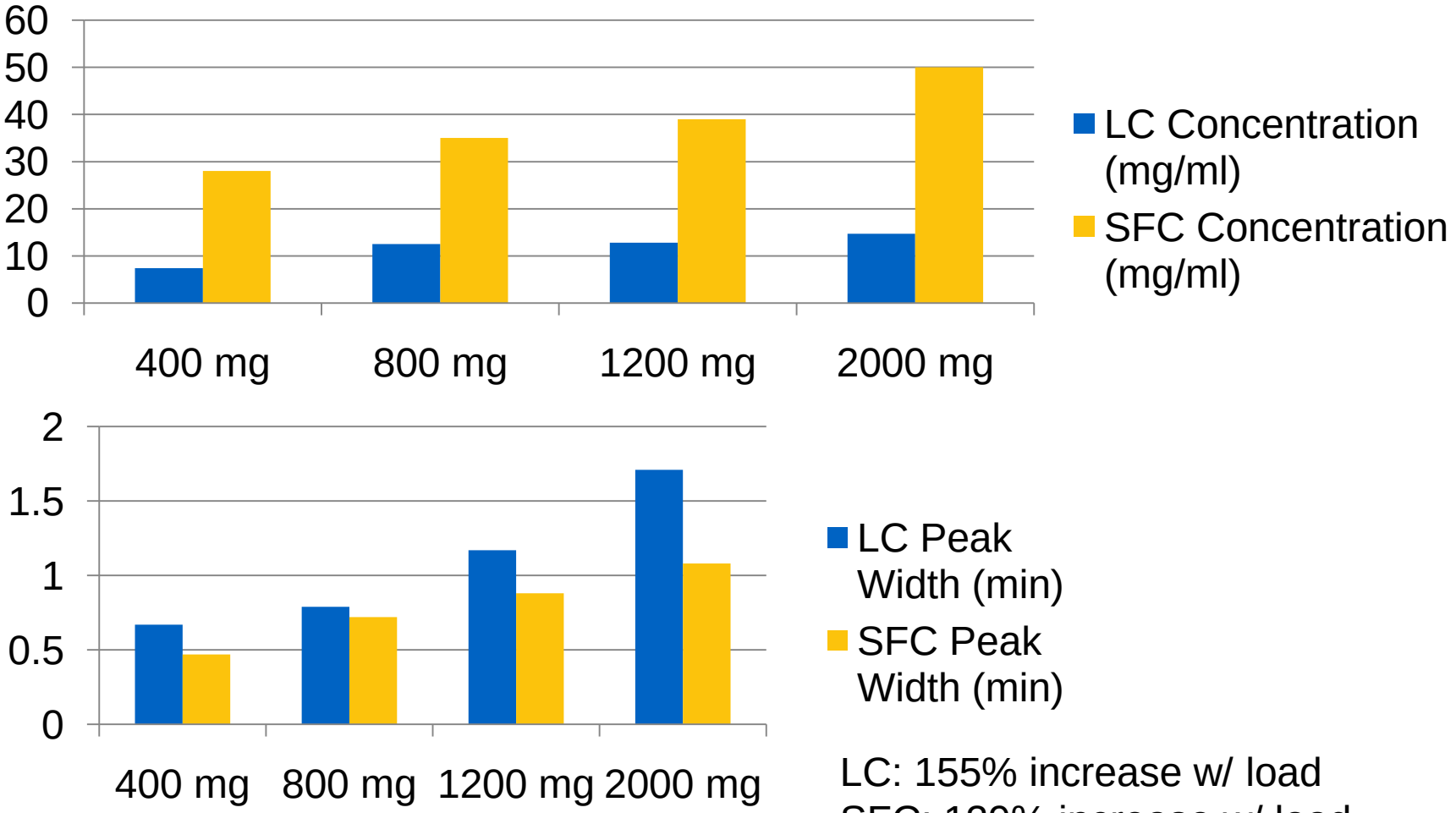


Mixture 3: Flash LC/SFC Comparison

Technique	Load (mg)	Peak 1 Volume	Peak 2 Volume	Solvent (L/g crude)	Productivity (g/hr)*
LC	400	66	27	1.85	0.84
LC	800	50	32	0.93	1.68
LC	1200	69	47	0.62	2.52
LC	2000	90	68	0.37	4.2
SFC	400	4.5	7.1	0.56	1.68
SFC	800	7.6	11.5	0.28	3.36
SFC	1200	10.0	15.5	0.19	5.04
SFC	2000	12.5	19.9	0.11	8.4

* Assume 10 minute re-equilibration for LC, 5 minutes for SFC

Mixture 3: Peak 2 LC/SFC Comparison



LC: 155% increase w/ load
SFC: 129% increase w/ load

Summary

- TLC R_f to SFC R_t correlations are not strong
- Dry load injection gives superior loading and peak shape relative to “solvent” injection techniques
- High loadings are possible with flash SFC
- Comparable loadings are obtained for flash LC and flash SFC
- Flash SFC has potential advantages over flash LC for med chem purifications but significant additional work needs to be performed.

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AMGEN